

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

19036/37157

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/763712

INTERNATIONAL APPLICATION NO.

PCT/JP99/04552

INTERNATIONAL FILING DATE

24 August 1999

PRIORITY DATE CLAIMED

24 August 1998

TITLE OF INVENTION

NOVEL COLLECTIN

APPLICANT(S) FOR DO/EO/US

WAKAMIYA, Nobutaka

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IEPA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

## \* Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Computer Readable Copy of Sequence Listing

Statement Regarding Sequence Listing Pursuant to 37 C.F.R. 1.825 (a) and 1.825(b)

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PCT/JP99/04552

19036/37157

21. The following fees are submitted:

**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :**

- ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... \$1,000.00
- ☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... \$860.00
- ☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$710.00
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$690.00
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$100.00

**ENTER APPROPRIATE BASIC FEE AMOUNT =**

\$860.00

Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). ☐ 20 ☐ 30

\$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =	0	x \$18.00	\$0.00
Independent claims	- 3 =	0	x \$80.00	\$0.00
Multiple Dependent Claims (check if applicable)			<input type="checkbox"/>	\$0.00

**TOTAL OF ABOVE CALCULATIONS =** \$860.00

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable). ☐

\$0.00

**SUBTOTAL =** \$860.00

Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). ☐ 20 ☐ 30 +

\$0.00

**TOTAL NATIONAL FEE =** \$860.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). ☐

\$0.00

**TOTAL FEES ENCLOSED =** \$860.00

Amount to be:	\$
refunded	
charged	\$

- ☒ A check in the amount of \$860.00 to cover the above fees is enclosed.
- ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.
- ☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 13-2855 A duplicate copy of this sheet is enclosed.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

HOPKINS, Mark H.  
MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BORUN  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
U.S.

SIGNATURE

Mark H. Hopkins

NAME

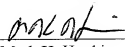
44,775

REGISTRATION NUMBER

DATE

PATENT  
19036/37157

IN THE UNITED STATES  
PATENT AND TRADEMARK OFFICE

In re Application of: Wakamiya, N.	)	CERTIFICATE OF MAILING BY
	)	EXPRESS MAIL
Serial No.: To Be Determined	)	"EXPRESS MAIL" mailing label
	)	No. <u>EL564457185US</u>
Filed: Herewith	)	Date of Deposit: February 26, 2001
(US National Phase of PCT/JP99/04552,	)	
Filed 24 August 1999)	)	I hereby certify that this paper and the
	)	documents referred to herein as enclosed
Title: "Novel Collectin"	)	herewith are being deposited with the
	)	United States Postal Service "EXPRESS
Group Art Unit: To Be Determined	)	MAIL POST OFFICE TO
	)	ADDRESSEE" service under 37 C.F.R.
Examiner: To Be Determined	)	\$1.10 on the date indicated above and
	)	are addressed to Box PCT,
Docket No: 19036/37157	)	Commissioner for Patents, Washington,
	)	D.C. 20231.
	)	
	)	Mark H. Hopkins

**PRELIMINARY AMENDMENT "A"**

BOX PCT  
Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

Please amend the above-identified patent application as follows before  
calculating the filing fee and before examination on the merits.

## Amendments

### In the specification:

Please delete sequence listing pages 1-17 of the translation of the specification filed herewith, which constitute a translation of the sequence listing, and substitute therefore new pages 1-11 appended hereto, which constitute a substitute Sequence Listing.

Please replace the paragraph beginning at page 1, line 13, with the following amended paragraph:

-- The collectins that have been identified heretofore include mannan-binding protein (MBP, SEQ ID NO: 30), surfactant protein A (SP-A, SEQ ID NO: 31), surfactant protein D (SP-D, SEQ ID NOS: 32), conglutinin and the like. These collectins are known to be constituted from basic structures (Fig. 1) comprising unique regions of: (1)  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and (2) collagen-like region [Malhortra *et al.*, *Eur. J. Immunol.* Vol. 22, 1437-1445, 1992], and a subunit may be formed from the three basic structures through making a triple helix in the collagen-like region, besides, such subunits may form an oligomer, e.g. trimer, tetramer and hexamer.--

Please replace the paragraphs at page 3, lines 16-27, with the following amended paragraphs:

-- Figure 2 shows the alignment of the preceding half portions of amino acid sequences (SEQ ID NOS: 30-32) of three collectins reported heretofore;

Figure 3 shows the alignment of the latter half portions of the amino acid sequences (SEQ ID NOS: 30-32) in Figure 2;

Figure 4 shows each of the primers used for sequencing the novel collectin of the present invention, and the nucleotide sequences which were read out from the sequencer (b); and an ORF of the obtained novel collectin (a);

Figure 5 shows the alignment of the preceding half portions of amino acid sequences (SEQ ID NOS: 30-32) of the three collectins reported heretofore and the novel collectin of the present invention (SEQ ID NO: 2, residues 206-547);

Figure 6 shows the alignment of the latter half portions of the amino acid sequences (SEQ ID NOS: 2 and 30-32) in Figure 5;--

New Abstract of the disclosure:

Please amend the application by replacing the Abstract of the Disclosure on page 64 of the translation of the application filed herewith with the attached Abstract of the Disclosure. Please replace the attached Abstract of the Disclosure after the claims and prior to the drawing sheets.

**Remarks/Arguments**

The Applicants request entry of the foregoing amendments to correct the Sequence Listing filed as part of the specification in the above-identified application. Accompanying this amendment is a substitute Sequence Listing in both computer-readable and paper forms, along with a statement that the disclosures in the computer-readable and paper forms are the same and do not introduce new matter into the disclosure of the application.

As explained in the accompanying statement, SEQ ID NOS: 1-29 of the original and substitute Sequence Listings are identical. Thus, these sequences do not introduce new matter.

SEQ ID NOS: 30 (Mannan-Binding Protein, MBP), 31 (Surfactant protein A, SP-A) and 32 (Surfactant protein D, SP-D) of the substitute Sequence Listing do not introduce new matter into the disclosure of the application. These sequences are fully supported in Figures 2-3 and Figures 5-6 of the specification as originally filed. The substitute Sequence Listing has been prepared with the Patent Office's preferred PatentIn software and is accompanied by the requisite computer-readable copy and statement.

The amendments to the specification merely introduce appropriate cross-references to the sequence listing, consistent with U.S. practice guidelines.

The replacement Abstract of the disclosure is identical to the abstract found on the cover of the published PCT application from which the present application is derived, and it finds support throughout the application.


The applicant intends to file a subsequent preliminary amendment to amend the claims to improve grammar, place the claims in more conventional U.S. form, and minimize the filing fee by eliminating multiple dependencies. The applicants will pay any necessary fees for extra claims at that time.

Also attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made."

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402

Date: February 26, 2001

  
\_\_\_\_\_  
Mark H. Hopkins  
Reg. No. 44,775

ABSTRACT

Novel collectin-related molecules expected as exerting antibacterial and antiviral activities, etc. particularly in the human body, namely, a novel collectin gene containing the base sequence represented by SEQ ID NO:1 and novel collectin containing the amino acid sequence represented by SEQ ID NO:2; and a method with the use of the same.

**In the specification:**

Please replace the paragraph beginning at page 1, line 13, with the following amended paragraph:

-- The collectins that have been identified heretofore include mannan-binding protein (MBP, SEQ ID NO: 30), surfactant protein A (SP-A, SEQ ID NO: 31), surfactant protein D (SP-D, SEQ ID NOS: 32), conglutinin and the like. These collectins are known to be constituted from basic structures (Fig. 1) comprising unique regions of: (1)  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and (2) collagen-like region [Malhortra *et al.*, *Eur. J. Immunol.* Vol. 22, 1437-1445, 1992], and a subunit may be formed from the three basic structures through making a triple helix in the collagen-like region, besides, such subunits may form an oligomer, e.g. trimer, tetramer and hexamer.--

Please replace the paragraphs at page 3, lines 16-27, with the following amended paragraphs:

-- Figure 2 shows the alignment of the preceding half portions of amino acid sequences (SEQ ID NOS: 30-32) of three collectins reported heretofore;

Figure 3 shows the alignment of the latter half portions of the amino acid sequences (SEQ ID NOS: 30-32) in Figure 2;

Figure 4 shows each of the primers used for sequencing the novel collectin of the present invention, and the nucleotide sequences which were read out from the sequencer (b); and an ORF of the obtained novel collectin (a);

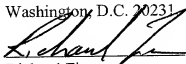
Figure 5 shows the alignment of the preceding half portions of amino acid sequences (SEQ ID NOS: 30-32) of the three collectins reported heretofore and the novel collectin of the present invention (SEQ ID NO: 2, residues 206-547);

Figure 6 shows the alignment of the latter half portions of the amino acid sequences (SEQ ID NOS: 2 and 30-32) in Figure 5;--



PATENT  
19036/37157

IN THE UNITED STATES  
PATENT AND TRADEMARK OFFICE

In re Application of: Wakamiya, N.	)	CERTIFICATE OF MAILING BY
	)	EXPRESS MAIL
Serial No.: 09/763,712	)	"EXPRESS MAIL" mailing label
	)	No. <u>EL827655848US</u>
Filed: Feb. 26, 2001	)	
(US National Phase of PCT/JP99/04552,	)	Date of Deposit: <u>May 4, 2001</u>
Filed 24 August 1999)	)	
Title: "Novel Collectin"	)	I hereby certify that this paper and the
	)	documents referred to herein as enclosed
Group Art Unit: To Be Determined	)	herewith are being deposited with the
	)	United States Postal Service "EXPRESS
Examiner: To Be Determined	)	MAIL POST OFFICE TO
	)	ADDRESSEE" service under 37 C.F.R.
Docket No: 19036/37157	)	§1.10 on the date indicated above and
	)	are addressed to the Commissioner for
	)	Patents, Box PCT
	)	Washington, D.C. 20231
	)	
	)	Richard Zimmermann

**PRELIMINARY AMENDMENT "B"**

BOX PCT  
Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

Please amend the above-identified patent application as follows before  
calculating the filing fee and before examination on the merits.

## Amendments

### Sequence Listing:

Please delete the sequence listing as originally filed and substitute therefore the substitute sequence listing filed herewith.

### In the claims:

Please cancel claims 1-37.

Please add claims 38-96 as shown below.

38. An isolated polynucleotide comprising a nucleotide sequence that encodes a polypeptide having an amino acid sequence that comprises positions 229-547 of SEQ ID NO: 2.

39. An isolated polynucleotide according to claim 38, comprising a nucleotide sequence set forth in positions 739-1695 of SEQ ID NO: 1.

40. An isolated polynucleotide according to claim 38, comprising a nucleotide sequence set forth in positions 739-2024 of SEQ ID NO: 1.

41. An isolated polynucleotide according to claim 38 wherein the amino acid sequence comprises positions 206-547 of SEQ ID NO: 2.

42. An isolated polynucleotide according to claim 41, comprising a nucleotide sequence set forth in positions 670-1695 of SEQ ID NO: 1.

43. An isolated polynucleotide according to claim 41, comprising a nucleotide sequence set forth in positions 670-2024 of SEQ ID NO: 1.

44. The polynucleotide according to claim 38, wherein said amino acid sequence comprises positions 226-547 of SEQ ID NO: 2.

45. An isolated polynucleotide according to claim 44, comprising a nucleotide sequence set forth in positions 730-1695 of SEQ ID NO: 1.

46. An isolated polynucleotide according to claim 44, comprising a nucleotide sequence set forth in positions 730-2024 of SEQ ID NO: 1.

47. The polynucleotide according to claim 38, wherein said amino acid sequence comprises positions 211-547 of SEQ ID NO: 2.

48. An isolated polynucleotide according to claim 47, comprising a nucleotide sequence set forth in positions 685-1695 of SEQ ID NO: 1.

49. An isolated polynucleotide according to claim 47, comprising a nucleotide sequence set forth in positions 685-2024 of SEQ ID NO: 1.

50. The polynucleotide according to claim 38, wherein said amino acid sequence comprises an amino acid sequence selected from the group consisting of: positions 102-547 of SEQ ID NO: 2; positions 91-547 of SEQ ID NO: 2; positions 9-547 of SEQ ID NO: 2; and positions 1-547 of SEQ ID NO: 2.

51. An isolated polynucleotide according to claim 38, comprising a nucleotide sequence selected from the group consisting of: positions 358-1695 of SEQ ID NO: 1; positions 325-1695 of SEQ ID NO: 1; positions 79-1695 of SEQ ID NO: 1; positions 55-1695 of SEQ ID NO: 1; and positions 1-1695 of SEQ ID NO: 1.

52. An isolated polynucleotide according to claim 38, comprising a nucleotide sequence selected from the group consisting of: positions 358-2024 of SEQ ID NO: 1;

positions 325-2024 of SEQ ID NO: 1; positions 79-2024 of SEQ ID NO: 1; positions 55-2024 of SEQ ID NO: 1; and positions 1-2024 of SEQ ID NO: 1.

53. An isolated polynucleotide encoding a collectin, which hybridizes under stringent conditions with a probe that is an amplification product from a PCR reaction performed using primers having the following nucleotide sequences: caatctgatgagaaggtgatg (SEQ ID NO: 4) and acgaggggctggatgggacat (SEQ ID NO: 5).

54. An isolated nucleic acid which can hybridize under stringent conditions with the polynucleotide according to claim 38, wherein the nucleic acid encodes a collectin which comprises a  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and a collagen-like region.

55. An isolated nucleic acid which can hybridize under stringent conditions with the polynucleotide according to claim 39, wherein the nucleic acid encodes a collectin which comprises a  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and a collagen-like region.

56. An isolated nucleic acid which can hybridize under stringent conditions with the polynucleotide according to claim 51, wherein the nucleic acid encodes a collectin which comprises a  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and a collagen-like region.

57. The polynucleotide according to claim 38, wherein said polynucleotide is cDNA.

58. An isolated collectin polypeptide comprising the amino acid sequence encoded by the polynucleotide according to claim 53.

59. An isolated collectin polypeptide comprising the amino acid sequence encoded by the polynucleotide according to claim 54.

60. An isolated collectin polypeptide comprising the amino acid sequence encoded by the polynucleotide according to claim 56.

61. An isolated collectin polypeptide comprising an amino acid sequence set forth in positions 229-547 of SEQ ID NO: 2.

62. An isolated collectin polypeptide according to claim 61 comprising an amino acid sequence set forth in positions 206-547 of SEQ ID NO: 2.

63. The collectin polypeptide according to claim 61, wherein said amino acid sequence comprises positions 226-547 of SEQ ID NO: 2.

64. The collectin polypeptide according to claim 61, wherein said amino acid sequence comprises positions 211-547 of SEQ ID NO: 2.

65. The collectin polypeptide according to claim 61, wherein said amino acid sequence comprises a sequence selected from the group consisting of: positions 102-547 of SEQ ID NO: 2, positions 91-547 of SEQ ID NO: 2, positions 9-547 of SEQ ID NO: 2, and positions 1-547 of SEQ ID NO: 2.

66. An isolated human collectin polypeptide according to claim 58.

67. An isolated human collectin polypeptide according to claim 59.

68. An isolated human collectin polypeptide according to claim 60.

69. An isolated human collectin polypeptide according to claim 61.

70. The collectin according to claim 61 that comprises deletion, substitution and/or addition of one or more amino acids in the amino acid sequence and that comprises a  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD) and a collagen-like region.

71. A vector which comprises the polynucleotide according to claim 38 and that allows the expression of the amino acid sequence encoded by the polynucleotide.

72. A vector which comprises the polynucleotide according to claim 50 and that allows the expression of the amino acid sequence encoded by the polynucleotide.

73. A vector which comprises the polynucleotide according to claim 53 and that allows the expression of the collectin encoded by the polynucleotide.

74. A vector which comprises the nucleic acid according to claim 55 and that allows the expression of the collectin encoded by the polynucleotide.

75. A vector which comprises the nucleic acid according to claim 56 and that allows the expression of the collectin encoded by the polynucleotide.

76. A host cell comprising the vector according to claim 71 that expresses the amino acid sequence encoded by the polynucleotide.

77. A host cell comprising the vector according to claim 72 that expresses the amino acid sequence encoded by the polynucleotide.

78. A host cell comprising the vector according to claim 73 that expresses the collectin.

79. A host cell comprising the vector according to claim 74 that expresses the collectin.

80. A host cell comprising the vector according to claim 75 that expresses the collectin.

81. A probe for screening for a collectin homolog comprising the polynucleotide according to claim 38 or a fragment thereof.

82. An antibody having specific immunoreactivity with a polypeptide comprising an amino acid sequence selected from the group consisting of: positions 229-547 of SEQ ID NO: 2; positions 226-547 of SEQ ID NO: 2; positions 211-547 of SEQ ID NO: 2; positions 206-547 of SEQ ID NO: 2; positions 102-547 of SEQ ID NO: 2; positions 91-547 of SEQ ID NO: 2; positions 9-547 of SEQ ID NO: 2; and positions 1-547 of SEQ ID NO: 2.

83. The antibody according to claim 82 which is a monoclonal antibody.

84. The antibody according to claim 82 having reduced immunogenicity to a human.

85. A method for obtaining a collectin homolog comprising screening for proteins that bind the antibody according to claim 82, and isolating a protein bound by the antibody.

86. The method according to claim 85, wherein said screening step comprises expressing a cDNA library and screening proteins encoded thereby.

87. A method of quantitative determination of a collectin and/or a homolog comprising contacting a sample suspected of comprising a collectin or homolog thereof with an antibody according to claim 82, and measuring an amount of the collectin and/or homolog contained therein from the quantity of antibody bound thereto.

88. The method according to claim 87, wherein said antibody is employed in an ELISA method.

89. An ELISA kit for quantitative determination of a collectin, comprising the antibody according to claim 82.

90. A method for isolating a collectin and/or a homolog thereof, comprising a step of contacting a sample that contains the collecting or homolog with the antibody according to claim 82.

91. The method according to claim 90, wherein the antibody is bound to a solid support, and wherein the contacting step comprises contacting the sample with the antibody to immunoprecipitate the collectin or homolog.

92. The method according to claim 90, wherein the antibody is bound to a solid support, and wherein the contacting step comprises an affinity chromatography step using the antibody bound to the solid support.

93. A method for making a collectin polypeptide, comprising steps of:  
expressing a polynucleotide according to claim 38 that encodes a collectin polypeptide comprising an amino acid sequence selected from the group consisting of: positions 229-547 of SEQ ID NO: 2; positions 226-547 of SEQ ID NO: 2; positions 211-547 of SEQ ID NO: 2; positions 206-547 of SEQ ID NO: 2; positions 102-547 of SEQ ID NO: 2; positions 91-547 of SEQ ID NO: 2; positions 9-547 of SEQ ID NO: 2; and positions 1-547 of SEQ ID NO: 2; and

purifying the collectin polypeptide using an antibody according to claim 82.

94. A non-human transgenic animal comprising a recombinant gene that comprises a polynucleotide according to claim 38, wherein the polynucleotide is stably integrated into a chromosome of the animal, and wherein the animal expresses the amino acid sequence encoded by the polynucleotide.



95. A transgenic non-human animal wherein introduction of a gene is effected in a non-human animal comprising a homologue of the collectin polypeptide according to claim 61, in a manner to modify the gene expression of the homologue.

96. A knockout, non-human animal that is obtained by altering a gene encoding a homologue of the novel collectin polypeptide according to claim 61 in a non-human animal comprising said homologue, in a manner to inhibit the expression of said homologue.

### **Remarks/Arguments**

The Applicants request entry of the foregoing amendments to correct the Sequence Listing filed as part of the specification in the above-identified application. Accompanying this amendment is a substitute Sequence Listing in both computer-readable and paper forms, along with a statement that the disclosures in the computer-readable and paper forms are the same and do not introduce new matter into the disclosure of the application.

As explained in the accompanying statement, SEQ ID NOS: 1-29 of the original and substitute Sequence Listings are identical. Thus, these sequences do not introduce new matter.

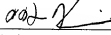
SEQ ID NOS: 30 (Mannan-Binding Protein, MBP), 31 (Surfactant protein A, SP-A) and 32 (Surfactant protein D, SP-D) of the substitute Sequence Listing do not introduce new matter into the disclosure of the application. These sequences are fully supported in Figures 2-3 and Figures 5-6 of the specification as originally filed. The substitute Sequence Listing has been prepared with the Patent Office's preferred PatentIn software and is accompanied by the requisite computer-readable copy and statement.

The cancellation and addition to the claims are merely intended to improve grammar, place the claims in more conventional U.S. form, and minimize the filing fee by eliminating multiple dependancies and are not intended to diminish the scope of the claims. The Applicant does not intend by these or any other amendments to abandon the subject matter of any claim as originally filed, and reserves the right to pursue such subject matter in this application or related applications, such as continuing applications.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402

Date: May 4, 2001



---

Mark H. Hopkins  
Reg. No. 44,775

Novel Collectin

## [Field of the Invention]

The present invention relates to a novel collectin which is useful for investigating mechanisms of biological defense system, and is expected to be applied for utilizing as materials for medicines because it may have physiological activities including anti-viral activities and the like.

## [Background Art]

Collectin is a generic name of proteins having  $\text{Ca}^{2+}$ -dependent carbohydrate recognition region (CRD) and collagen-like region, and the member of these proteins is conceived to involve in basic immunity systems against a wide spectrum of microorganisms such as bacteria and viruses.

The collectins that have been identified heretofore include mannan-binding protein (MBP), surfactant protein A (SP-A), surfactant protein D (SP-D), conglutinin and the like. These collectins are known to be constituted from basic structures (Fig. 1) comprising unique regions of: (1)  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and (2) collagen-like region [Malhorta *et al.*, *Eur.J.Immunol.* Vol.22, 1437-1445, 1992], and a subunit may be formed from the three basic structures through making a triple helix in the collagen-like region, besides, such subunits may form an oligomer, e.g., trimer, tetramer and hexamer.

In vertebrates, mechanisms involving cellular immune responses and specific antibody reactions are considered as dominant host-defense systems against invasion of the pathogenic bacteria, viruses and the like. Recently, involvement in nonspecific immune responses of the lectins such as conglutinin has been suggested, for example, it was reported that the lectins may play important roles in neutralizing and removing the various microorganisms in infants having insufficient maternal antibodies and undeveloped specific defense systems [Super *et al.*, *Lancet*, Vol.2, 1236-1239, 1989].

Moreover, with respect to the roles of the lectins in the biological host-defense systems, it was reported that the host becomes liable to be infected by, for example, a reduction of the MBP concentration in blood due to genetic mutation of the MBP gene [Sumiya *et al.*, *Lancet*, Vol.337, 1569-1570, 1991].

The present inventors previously found that the conglutinin and the mannan-binding protein can inhibit infection and hemagglutination activity of H1 and H3

Type Influenza A viruses [Wakamiya et al., *Glycoconjugate J.*, Vol.8, 235, 1991; Wakamiya et al., *Biochem. Biophys. Res. Comm.*, Vol.187, 1270-1278, 1992].

Thereafter, the present inventors isolated a cDNA clone encoding the conglutinin, and found that closer correlation may exist between the conglutinin gene and various surfactant protein D gene [Suzuki et al., *Biochem. Biophys. Res. Comm.*, Vol.191, 335-342, 1993].

Thus, the collectin has been expected to be useful in investigating mechanisms of biological defense, and useful as a physiologically active medical material. Therefore, discovery of novel molecular species belonging to this family would largely contribute to various medical fields including therapy of infectious diseases, as well as biological fields.

#### [Disclosure of the Invention]

The present invention was accomplished in consideration of the aforementioned state of art, and an object of the invention is to provide a novel collectin which can be expected to exhibit physiological activities such as anti-bacterial, anti-viral activities, especially in human body.

Accordingly, the present invention provides novel collectin gene and protein having characteristic structures of the collectins, which are distinct from those reported heretofore as follows:

[1] A polynucleotide comprising a nucleotide sequence which encodes a protein having an amino acid sequence set out in SEQ ID NO: 2, 206-547;

[2] A polynucleotide comprising a nucleotide sequence which encodes a protein having an amino acid sequence set out in SEQ ID NO: 2, 229-547;

[3] A polynucleotide comprising a nucleotide sequence set out in SEQ ID NO: 1, 670-1695;

[4] A polynucleotide comprising a nucleotide sequence set out in SEQ ID NO: 1, 739-1695;

[5] A polynucleotide encoding a collectin protein, wherein said polynucleotide can hybridize under a stringent condition with a probe that is an amplification product of PCR reaction conducted using primers having nucleotide sequences set out in SEQ ID NO: 4 and SEQ ID NO: 5;

[6] A polynucleotide which can hybridize under a stringent condition with any one of the polynucleotide described in [1] to [5], wherein the protein encoded by said

polynucleotide is a human collectin comprising (1)  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and (2) collagen-like region;

[7] A novel collectin encoded by the polynucleotide of [3] to [6];

[8] A novel collectin comprising an amino acid sequence set out in SEQ ID NO:2, 206-547;

[9] A novel collectin comprising an amino acid sequence set out in SEQ ID NO:2, 229-547;

[10] The novel collectin according to any one of [7] to [9] consisting of the amino acid sequence that comprises deletion, substitution and/or addition of one or more amino acids, and wherein the amino acid sequence thereof comprises those corresponding to (1)  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and (2) collagen-like region.

#### [Brief Description of the Drawings]

Figure 1 is a schematic drawing showing basic structures and overviews of the principal collectins reported heretofore;

Figure 2 shows the alignment of the preceding half portions of amino acid sequences of three collectins reported heretofore;

Figure 3 shows the alignment of the latter half portions of the amino acid sequences in Figure 2;

Figure 4 shows each of the primers used for sequencing the novel collectin of the present invention, and the nucleotide sequences which were read out from the sequencer (b); and an ORF of the obtained novel collectin (a);

Figure 5 shows the alignment of the preceding half portions of amino acid sequences of the three collectins reported heretofore and the novel collectin of the present invention;

Figure 6 shows the alignment of the latter half portions of the amino acid sequences in Figure 5;

Figure 7 shows results of genomic Southern analysis with the novel collectin of the present invention, and the restriction enzymes employed in each of the lanes are (1) EcoRI, (2) XbaI, (3) HindIII, (4) PstI, (5) BglIII and (6) BamHI;

Figure 8 shows results of analysis of distribution of expression of mRNA in: (1) brain, (2) heart, (3) kidney, (4) spleen, (5) liver, (6) small intestine, (7) muscular tissue, (8) testis, (9) placenta, or (10) large intestine demonstrating the tissue distribution of the

novel collectin of the present invention;

Figure 9 shows results of genomic Southern analysis of various vertebrates, i.e., (1) human, (2) monkey, (3) rat, (4) mouse, (5) dog, (6) cow, (7) rabbit and (8) chicken to elucidate the interspecies conservation of the novel collectin of the present invention;

Figure 10 shows a phylogenetic tree of various collectins; and

Figure 11 is schematic drawings illustrating a vector that expresses the novel collectin of the present invention.

#### [Best Mode for Carrying Out the Invention]

In the preferred embodiment of the present invention, the above-mentioned polynucleotide in any of [1] to [6] of the present invention may be preferably cDNA.

The polynucleotide in the above [2] comprises a nucleotide sequence encoding a protein having at least an amino acid sequence set out in SEQ ID NO: 2, 229-547, however, additional nucleotide sequence such as those encoding a protein having an amino acid sequence such as 226-228 or 211-228 of SEQ ID NO: 2, or 102-228, 91-228, 9-228, 1-228 of SEQ ID NO: 2 may be contained upstream of the first methionine residue.

Moreover, the polynucleotide set out in the above [4] may further comprise a nucleotide sequence of 730-738 or 685-738, or 358-738, 325-738, 79-738, 55-738 or 1-738 in SEQ ID NO: 1, 5' upstream thereof.

Additionally, the polynucleotide in the above [3] or [4] may comprise a nucleotide sequence of 1696-204 in SEQ ID NO:1, 3' downstream thereof.

Further, the protein [7] to [10] of the present invention mentioned above may be preferably derived from human taking into account of the usefulness as physiologically active medical materials, because it can be expected to exhibit anti-bacterial, anti-viral activities and the like in human body. Accordingly, the present invention contemplates a novel collectin derived from human, and upon examination of various human tissues, expression of a novel collectin was suggested, which was conceived to be useful.

Further, the novel collectin of the above [9] may preferably comprise at least an amino acid sequence of 229-547 in SEQ ID NO: 2, and an amino acid sequence for example, 226-228 or 211-228 in SEQ ID NO: 2, or 102-228, 91-228, 9-228, 1-228 in SEQ ID NO: 2 or the like may be additionally included upstream of the first methionine residue.

The stringent hybridization condition in the inventions of the above [5] and [6] may include for example, a series of the following steps for the hybridization: prehybridization in a solution of 5 x SSC (prepared by diluting 20 x SSC (3 M NaCl, 0.3 M sodium citrate)), 1% blocking agent (Boehringer Mannheim), 0.1% N-lauroyl sarcosine, and 0.02% SDS, at 68°C for an hour; hybridization in a solution of 5 x SSC, 1% blocking agent, 0.1% N-lauroyl sarcosine, and 0.02% SDS containing cDNA probes (10 ng/ml), at 55°C for 16 hours; washing in a solution of 2 x SSC/0.1% SDS for 5 minutes, 2 times; and washing in a solution of 0.5 x SSC/0.1% SDS at 55°C for 15 minutes, 2 times. However, several modifications/alterations of these conditions such as the concentration of the solution, incubation temperature and time may be made on the basis of the knowledge of the skilled art.

Further, deletion, substitution and/or addition of one or more amino acids in the above [10] may be those which does not result in great changes of hydrophilic/hydrophobic, or acidic/basic nature of the amino acid residues of the novel collectin, without bringing much alteration of each of the properties of (1)  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD) and (2) collagen-like region. On the basis of the amino acid sequences and structures of the proteins belonging to the collectin family reported heretofore, for example, deletion, substitution and/or addition of 1-10 amino acid residues in (1)  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and 1-100, preferably 1-15 amino acid residues in (2) collagen-like region may be allowed.

The present invention also provides a vector, which comprises the above-described polynucleotide in a manner that allows expression of the novel collectin.

The vector in which the polynucleotide is inserted may be referred to as a replicon such as plasmid,  $\lambda$ -phage, cosmid and the like with the above described novel collectin polynucleotide being incorporated therein so that the novel collectin can be replicated and expressed. For example, vectors capable of cloning longer DNA fragments than cosmid may include P1 phage, F factor and artificial chromosome YAC of yeast and the like. Moreover,  $\lambda$ -phage may include replacement vector and insertion vector, and these can be selected ad libitum taking into account of the length of gene to be introduced. Moreover, known vectors capable of being expressed in animal cells include for example, SV40 vectors, bovine papilloma virus vector, herpes virus vector, adenovirus vector, poxvirus vector, retrovirus vector and the like. Commercially available vectors may include pUC19, pTV118N (Takara Shuzo Co., Ltd.), pUEX2 (Amersham), pGEX-4T, pKK233-2

(Pharmacia), pMAM-neo (Clontech), pGL2 (Promega), pDNA3.1+ (Invitrogen) and the like. A method of constructing such vectors can be performed utilizing restriction enzyme, ligase and the like by the conventional method without any particular limitation.

In the case where bacteria, in particular *E. coli*, are used as a host cell that is transformed or transfected with the above described vector, the vector may usually comprise at least a promoter region (containing promoter, operator and Shine-Dalgarno sequence), initiation codon, a nucleotide sequence encoding the novel collectin, termination codon, terminator region and the like. In the case where yeast or an animal cells are used as a host, it is preferable that the vector includes at least promoter, initiation codon, nucleotide sequences encoding a signal peptide and the novel collectin, and termination codon. Moreover, enhancer sequence, 5'- and 3'-untranslated region of the novel collectin, splicing junction, polyadenylation site and selectable marker can be also inserted into the vector.

As a promoter incorporated into a vector of the present invention, conventionally well known SV40 (Simian Virus 40), SR $\alpha$ , cytomegalovirus (CMV) promoter, actin promoter, viral LTR (Long Terminal Repeat), for example, HTLV-1 LTR, HIV-LTR, Rous sarcoma virus LTR, herpes simplex tyrosine kinase virus promoter and the like can be used irrespective of whether it is a strong promoter or a weak promoter.

In the case where the expression is expected in eukaryotic cells of many cell types ranging from normal cells such as fibroblast, nerve cell, blood cell, parenchymal cell and the like to cancer cells, generally employed promoter may include cytomegalovirus, thymidine kinase (TK),  $\beta$ -actin and SV40 early gene promoter and the like. Since an enhancer is usually combined with a promoter sequence, it can be utilized as it is.

Moreover, in the case where cells and tissues in which the gene is introduced and expressed have been specified, a promoter specific for the cell can be selected. Furthermore, by combining these promoters in a homologous or heterologous manner, high expression can be expected, and an effect may be exerted that the novel collectin is stably obtained.

On the other hand, a promoter used in prokaryotic cells, PBAD, PL, *trc*, T7, SP6, T3, *lac* and the like may be included.

In principle, a promoter that can result in high expression of the novel collectin in the cells from prokaryotic cells can be appropriately selected and used as a promoter of the present invention, as long as the promoter is compatible with the host. Moreover, in



the case where cells and tissues in which the gene is introduced and expressed have been specified, a promoter specific for the cell can be selected. Furthermore, by combining these promoters in a homologous or heterologous manner, high expression can be expected, and an effect may be exerted that the novel collectin is stably obtained.

On the other hand, promoter used in yeast may include GAL1, AOX1, CUP1, PGK and the like.

A selectable marker incorporated in the above described vector for recovering only the intended vectors may include dihydrofolate reductase (hereinafter, abbreviated as DHFR) gene, methotrexate resistant gene, neo gene (G418 resistant gene) and the like, and for example, in the case where DHFR gene is used as a selectable marker employing CHO cell of which DHFR gene is deleted, the selection can be also performed by a thymidine free medium. In addition, when the gene is expressed in the cell by culturing in the medium at a higher methotrexate concentration and selecting resistant cells, the cell strain with higher expression can be also obtained.

The present invention also contemplates a host cell transformed or transfected with the vector thus constructed, which can express the novel collectin of the present invention, for example, animal cells, insect cells and microorganic cells (*E.coli*, *Bacillus subtilis*, yeast, and the like).

Animal cells used as the host cell of the present invention may include cells derived from human, but not limited thereto. For example, CHO cell, COS cell, BHK cell, Vero cell, myeloma cell, HEK 293 cell, HeLa cell, Jurkat cell, mouse L cell, mouse C127 cell, mouse FM3A cell, mouse fibroblast, osteoblast, cartilage cell and the like may be included. Moreover, as the microorganic cell, for example, bacteria such as *E. coli*, *Bacillus subtilis* and the like, and yeasts such as *Saccharomyces cerevisiae*, beer yeast and the like may be included.

Moreover, according to the present invention, a probe for screening a novel collectin related molecular species including the above polynucleotide or a fragment thereof is provided. The novel collectin related molecular species means a molecular species of a novel collectin having a characteristic structure as shown in FIG. 1, without being included in the known collectins shown in FIG. 10. Using a probe of the present invention, a desired novel collectin related molecular species can be acquired by examination of a hybridizing ability between the probe and a gene obtained from tissue cells and the like of a variety of organisms, screening a gene encoding the desired novel

collectin related molecular species through searching a gene that can hybridize with the probe under a relatively stringent condition. Among the above described fragments of the polypeptide, those comprising a nucleotide sequence of at least a stretch of 10 bases or more, preferably a stretch of 20 bases or more out of a sequence of 1291-1698 in SEQ ID NO: 1 that comprises a carbohydrate recognition domain structural region, or those comprising a nucleotide sequence of at least a stretch of 10 bases or more, preferably a stretch of 20 bases or more out of a sequence of 796-1236 in SEQ ID NO: 1 that comprises a collagen-like region may be useful as a polynucleotide for constituting the probe in terms of its specificity.

The present invention also provides an antibody that recognizes a novel collectin and/or derived molecules obtained according to the present invention. As referred to herein, the term "derived molecules" may include fragments having similar characteristics with the novel collectin (for example, fragments containing carbohydrate recognition domain and/or collagen-like region), precursors of the novel collectin, and polypeptides comprising amino acid sequence encoded by the nucleic acid which can hybridize under the stringent condition with the nucleotide sequence coding the fragments, precursors, and/or complementary nucleic acid sequence thereto.

An antibody (for example, polyclonal antibody, monoclonal antibody and peptide antibody) or anti-serum for the novel collectin and/or derived molecules can be manufactured using the present novel collectin and/or derived molecules, or fusion protein containing the same, and the like, as an antigen in accordance with known method of generating the antibody or anti-serum. Namely, the present novel collectin and/or derived molecules, or fusion protein containing the same may be administered as an antigen alone or in combination with a diluent or a carrier to the site in which antibody production is allowed through the administration to the warm-blooded animals. Upon administration, Freund's complete adjuvant or Freund's incomplete adjuvant may be administered in order to elevate the production of the antibody. The administration may be usually performed once per every 1-6 weeks, 2-10 times in total. The warm-blooded animals employed may include for example, monkey, rabbit, dog, guinea pig, mouse, rat, sheep, goat, chicken and the like, and mouse and rat are preferred. Among rats, Wistar and SD strain rat and the like are preferable, and among mice, BALB/c, C57BL/6 and ICR strain mouse and the like are preferably used.

When the monoclonal antibody producing cells are prepared, an individual that is

found with antibody titer is selected from the warm-blooded animal e.g., mouse, then spleen or lymph node is recovered in 2-5 days after the final immunization, followed by fusion of myeloma cells and the antibody producing cells contained therein to yield the monoclonal antibody producing hybridoma. The measurement of the antibody titer in anti-serum may be performed by, for example, the reaction of the anti-serum with a labeled novel collectin described below or the novel collectin, and thereafter determining the activity of the labeling agent coupled to the antibody. An operation for fusion can be carried out according to the known methods, for example, Kohler and Milstein's method (*Nature*, 256: 495, 1975) and a modified method thereof (*J. Immunol. Method.* 39: 285, 1980; *Eur. J. Biochem.*, 118: 437, 1981; *Nature*, 285: 446, 1980). As agents for promoting fusion, polyethylene glycol (PEG), Sendai virus and the like, preferably PEG may be employed. In order to further enhance fusion efficiency, lectin, poly-L-lysine or DMSO may be added ad libitum.

Myeloma cells may include for example, X-63Ag8, NS-1, P3U1, SP2/0, AP-1 cells and the like, however, SP2/0 cells may be preferably used. The suitable rate between the number of antibody producing cells (splenocyte) and the number of myeloma cells, which are used, may be 1:20-20:1, and cell fusion can be efficiently carried out by adding PEG (preferably PEG 1000-PEG 6000) at a concentration of approximately 10-80%, and incubating for 1-10 minutes at 20-40°C, preferably at 30-37°C. Although a variety of methods can be utilized for screening a hybridoma producing the antibody to the novel collectin and/or derived molecules, for example: a method in which hybridoma culture supernatant is added to a solid-phase (for example, microplate) where the novel collectin antigen is directly absorbed or along with a carrier, next, an anti-immunoglobulin antibody labeled by a radioactive substance, enzyme or the like, or protein A is added thereto, and the anti-novel collectin antibody coupled to the solid-phase is detected; a method in which hybridoma culture supernatant is added to the solid-phase where anti-immunoglobulin antibody or protein A is absorbed, followed by addition of the novel collectin labeled with a radioactive substance, enzyme or the like, and detection of the anti-novel collectin monoclonal antibody coupled to the solid-phase; and the like may be adopted.

Selection and cloning of the monoclonal antibody to the novel collectin and/or derived molecules may be performed according to a method known in the art or a method conformed thereto. In general, they may be performed in a selective medium for use in

animal cells to which HAT (hypoxanthine, aminopterin and thymidine) are added. As the medium for selection, cloning and growing, any medium may be employed as long as the hybridoma can grow therein. For example, RPMI medium containing 1-20%, preferably 10-20% of fetal bovine serum, GIT medium containing 1-10% of fetal bovine serum, a serum-free medium for use in hybridoma culture or the like can be employed. The temperature for culture may be preferably about 37°C. The time period of culture is usually, from 5 days to 3 weeks, preferably, from 1 week to 2 weeks. The culture is generally performed under the presence of air containing 5% carbon dioxide gas. The antibody titer of hybridoma culture supernatant can be measured in a similar manner to the procedure of the above-described measurement of the anti-novel collectin antibody titer in an the anti-serum. Specifically, as the method for measurement, radioimmunoassay (RIA) method, enzyme-linked immunosorbent assay (ELISA) method, fluorescence immunoassay (FIA) method, plaque assay method, coagulation reaction method and the like can be employed, however, the following ELISA method described below may be preferable.

A protein prepared by a similar procedure as in the case for immune antigen is immobilized on the surface of each well of ELISA plate. Next, for the purpose of preventing non-specific absorption, bovine serum albumin (BSA), mouse serum albumin (MSA), ovalbumin (OVA), gelatin, skim milk or the like are immobilized. Hybridoma culture supernatant is added to these respective wells, allowed to stand for a certain time period to effect immunoreaction. Each of the wells is washed using phosphate buffered saline (PBS) or the like as a wash solution. It is preferable to add a surface- active agent to the wash solution. An enzyme-labeled secondary antibody is added and allowed to stand for a certain time period. As the labeling enzyme,  $\beta$ -galactosidase, alkaline phosphatase, peroxidase and the like can be employed. After each well is washed with the same wash solution, a substrate solution for the used labeling enzyme is added, and enzyme reaction is effected. In the case where the intended antibody exists in the added hybridoma culture supernatant, the enzyme reaction is proceeded, and the color of the substrate solution is changed.

Cloning can be generally performed by a semi-solid agar method, a limiting dilution culture method and the like which are known in the art, in brief, after wells where the intended antibody is produced are determined, cloning is performed to obtain a single clone. As for a cloning method, the limiting dilution culture method may be utilized

wherein the hybridoma cells are diluted and cultured thereby forming one colony per well of culture plate. For cloning by the limiting dilution culture method, feeder cells may be employed, or cell growth factor such as interleukin 6 may be added in order to enhance colony forming ability. Alternatively, cloning can be performed using FACS (fluorescence-activated cell sorter) and a single cell manipulation method. The cloned hybridoma may be preferably cultured in a serum-free medium, and the optimal amount of the antibody is added to the supernatant. A single hybridoma thus obtained may be cultured in a large scale using flasks or a cell culture equipment, or otherwise, intraperitoneal culture in an animal is performed (*J. Immunol. Meth.*, 53:313, 1982), to allow for obtaining the monoclonal antibody. Culturing of the cells within a flask can be conducted using medium for cell culture (IMDM, DMEM, RPMI 1640, MEM and the like) containing 0-20% of fetal calf serum (FCS). When intraperitoneal culture in an animal is carried out, the animal of the same species, the same strain with the animal from which myeloma cell used for cell fusion is derived, athymic nude mouse or the like may be preferably used, and the hybridoma is transplanted after the previous administration of mineral oils such as pristan and the like. After 1-2 weeks, myeloma cells are grown in peritoneal, and ascites fluid containing the monoclonal antibody can be recovered.

A monoclonal antibody according to the present invention may be obtained as the one not cross-reacted with the other proteins by selecting the one that recognizes an epitope specific for the novel collectin and/or derived molecules. In general, an epitope presented by at least three or more serial amino acid residues, desirably amino acid sequence of 7-20 amino acids from the amino acid sequence constituting a protein is reported to show an epitope that is inherent in the protein. Therefore, a monoclonal antibody that recognizes an epitope constituted from the peptides selected from the amino acid sequences set forth in either of the novel collectin and/or derived molecules, and have amino acid sequences consisted of at least three serial amino acid residues may be referred to as a monoclonal antibody specific for a novel collectin and/or derived molecules of the present invention. When an amino acid sequence conserved between amino acid sequences set forth in the novel collectin and/or derived molecules is selected, a common epitope for the novel collectin family can be selected. Alternatively, when a region containing an amino acid sequence specific for the respective sequence is selected, a monoclonal antibody capable of distinguishing respective proteins can be selected.

Isolation and purification of a monoclonal antibody to the novel collectins and/or

derived molecules can be performed according to the isolation and purification method of immunoglobulin, similarly to the cases of the general polyclonal antibodies. As known purification methods, for example, salting-out method, alcohol precipitation method, isoelectric point precipitation method, electrophoresis method, ammonium sulfate precipitation method, absorption-desorption method by ion-exchanger (e.g., DEAE (diethylaminoethyl) Sepharose and the like), ultracentrifuge method, gel filtration method, a process of specific purification by collecting only an antibody with active absorbent such as antigen binding solid-phase, protein A, protein G or the like and dissociating the binding to obtain the antibody can be carried out. For the purpose of preventing the aggregate formation and reduction of the antibody titer during the purification process, for example, human serum albumin is added at a concentration of 0.05-2%. Besides, amino acids such as glycine,  $\alpha$ -alanine and the like, particularly, basic amino acids such as lysine, arginine, histidine and the like, sugars such as glucose, mannitol and the like or salts such as sodium chloride and the like may be added. IgM antibody is known to be especially liable to aggregate, therefore, treatment with  $\beta$ -propiolacton and acetic anhydride may be conducted.

A polyclonal antibody of the present invention can be manufactured according to the methods known in the art or methods conformed thereto. For example, immune antigen (protein antigen) itself, or a prepared complex thereof with a carrier protein, may be used to immunize a warm-blooded animal similarly to the above-described method of producing a monoclonal antibody, accordingly, the monoclonal antibody can be generated through collecting a material which contains an antibody to a protein or its fragments of the present invention from the immunized animal, and through isolation and purification of the antibody. In respect to the complex of the immune antigen and the carrier protein used for immunizing the warm-blooded animal, as for kind of the carrier protein as well as the mixing rate of the carrier and hapten, any kind of carrier protein may be crosslinked at any mixing ratio of the carrier and hapten as long as the antibody can be efficiently generated for the hapten crosslinked and immunized with the carrier. However, a method may be employed in which bovine serum albumin, bovine thyroglobulin, hemocyanin or the like is coupled with hapten at a weight ratio of about 0.1-20, preferably about 1-5 per hapten 1. Moreover, a variety of condensing agents can be used for coupling hapten and carrier, however, glutaraldehyde or carbodiimide, maleimide active esters, active ester reagents containing thiol group or dithiopyridyl group and the

like may be employed. The condensing product may be administered itself alone or in combination with a carrier or a diluent to a warm-blooded animal. Upon administration, Freund's complete adjuvant or Freund's incomplete adjuvant may be administered in order to enhance the antibody producing ability. The administration may be performed usually once every 2-6 weeks, about 3-10 times in total. A polyclonal antibody can be collected from the blood, ascites fluid and the like, preferably from blood of the warm-blooded animal that has been immunized by the above-described method. The measurement of the polyclonal antibody titer in the anti-serum can be performed similarly to the measurement of the antibody titer of the above-described serum. The isolation and purification of the polyclonal antibody can be performed according to the isolation and purification method of immunoglobulin, similarly to the above-described isolation and purification method of a monoclonal antibody.

Moreover, an antibody can be generated by using a fusion protein comprising the novel collectin and/or derived molecules and another protein or a polypeptide fragment as an immunogen. As another protein or the polypeptide constituting such a fusion protein, GST (glutathione-S-transferase) may be particularly suitable, besides, polyhistidine (preferably 6 histidine residues), calmodulin binding peptide (CBP), protein A and the like may be employed.

When polyhistidine is employed, absorption to nickel-chelating resin can be utilized for isolation and purification of the fusion protein expressed by a gene recombinant process, wherein addition of EDTA or imidazole substance as well as pH change may be adopted for dissociating the protein from the resin. When CBP is employed, the expressed fusion protein may be subjected to an affinity chromatography using calmodulin affinity resin, and then may be dissociated from the resin by adding EGTA. Further, when protein A is employed, the expressed fusion protein may be subjected to an affinity chromatography using IgG sepharose (e.g., IgG Sepharose 6FF), and thereafter may be dissociated from the resin by changing pH.

Moreover, another candidate for the protein or the polypeptide fragment to be employed in the fusion protein may include for example, Xpress, Thioredoxin, c-myc, V5, HA/c-myc and the like, and after the intended fusion protein with the novel collectin is expressed, it can be isolated and purified by an antigen-antibody affinity column using an antibody capable of recognizing such another protein as an epitope.

In the case where a monoclonal antibody is obtained using such fusion protein as

an immunogen, the novel collectin may be employed for screening the antibody, however, the screening may be preferably carried out with the fusion protein used as an immunogen in terms of the selectivity.

The aforementioned preferable immunogen, namely a fusion protein comprising GST and a novel collectin and/or derived molecules, may be suitably produced by: culturing *Escherichia coli* or animal cells transformed or transfected with an expression vector comprising a coding region of GST gene and a coding region of a novel collectin and/or derived molecules; and then isolating the fusion protein from the culture fluid and/or cells. Thus obtained fusion protein may be preferably purified further based on an affinity to a support carrying glutathione, e.g., glutathione sepharose beads, and a method of eluting the fusion protein from the glutathione sepharose beads can be performed by the known method. Accordingly, the purified fusion protein that is preferable as an intended immunogen can be obtained.

In a method of preparing a monoclonal antibody using such a fusion protein as an immunogen, a novel collectin and/or derived molecules may be employed for screening the antibody, however, it is more preferable to perform the screening using the fusion protein as an immunogen in terms of the specificity.

The monoclonal antibody and polyclonal antibody to the novel collectin and/or derived molecules can be utilized for diagnosis and treatment of diseases related to the novel collectin. It should be noted that in the case where the antibody is contemplated to administer particularly to a human body, the antibody such as humanized antibody or chimeric antibody, having no immunogenicity to human or having compromised immunogenicity as far as possible may be preferably used. When a mouse monoclonal antibody is administered into a human body, there may be several risks such that a variety of side effects may occur, because it is a heterologous protein to human. Hence, human monoclonal antibody would be desirable, however, fusion efficiency was poor, and to obtain a hybridoma that is stably producing the antibody was difficult. However, the technologies have been progressed so far, thus, generation of human monoclonal antibodies or chimeric antibodies has been possible at present.

The term "chimeric antibody" is referred to as a chimeric molecule of a mouse antibody and a human antibody. It is ethically impossible to manufacture an antibody by immunizing a given antigen to a human body. Therefore, a mouse is immunized, then a variable region (V region) of an antibody which may bind to an antigen is cut out from a



gene of the mouse monoclonal antibody, and a chimeric gene is prepared by coupling it to a constant region (C region) of an antibody gene derived from human myeloma cells. When this chimeric gene is expressed in a host cell, human/ mouse monoclonal antibody can be produced. Since a chimeric antibody has low antigenicity to human, it can be utilized as a monoclonal antibody to be administered into a human body for treatment, for diagnostic imaging and the like. With respect to known related technologies of chimeric antibodies, see, Japanese Unexamined Patent Publication No. H05-304989 (1993), Japanese Unexamined Patent Publication No. H04-330295 (1992), WO9106649, Japanese Unexamined Patent Publication No. S63-036786 (1988), and Japanese Patent Publication No. H06-98021 (1994) and the like.

However, a humanized antibody, which is reported to be more useful than a chimeric antibody has been recently developed. The term "humanized antibody" is an antibody, which is generated by grafting only a gene sequence of an antigen-binding site (CDR: complementary determining region) of an antibody molecule to a human antibody gene (CDR grafting), thus, the entire molecule is humanized except CDR of the antibody molecule. Since this antibody has less portions of mouse antibody than human/mouse chimeric antibody, it is believed to have less antigenicity and to be safer. In our country, a clinical trial of humanized antibody to adult T cell leukemia has been currently practiced. With respect to methods of manufacturing humanized antibody and related technologies thereof, see, Patent Applications such as Genentech (USA) (WO9222653, WO9845332, WO9404679, WO9837200, and WO9404679) and Celltech (UK) (WO9429451, WO9429351, WO9413805, WO9306231, WO9201059, WO9116927, WO9116928, WO9109967, WO8901974, and WO8901783) and the like.

Methods of generating a human monoclonal antibody may include: a method wherein transformation is effected with Epstein-Barr virus (EBV), followed by a method of fusing the transformed cells with parent cells; a method of preparing a chimeric antibody or a humanized antibody by utilizing genetic engineering; and the like, in addition to the cell fusion method. The chimeric antibody means an antibody prepared through joining immunoglobulin gene fragments of heterologous animals, and the humanized antibody means an antibody prepared through modification of an antibody of a heterologous antibody to human, such as that from mouse and the like, resulting in substitution of the primary structure with the corresponding primary structure of human except for a complementary determinant region (CDR) of H chain and L chain. As for

parent cells for use in the preparation of human monoclonal antibody, SHM-D 33 strain (ATCC CRL 1668) or RF-S1 strain, heteromyeloma of human/mouse, may be suitable employed to achieve a high fusion efficiency comparable to that of mouse parent cells. Thus obtained hybridoma using these parent cells can be subjected to cloning without feeder cells, and can produce an IgG type antibody in a comparatively stable manner at a large amount. ERDF medium to which 15% FCS is added may be employed for the culture of parent cells, and other procedures may be similarly carried out to those of the mouse. Moreover, in order to generate human monoclonal IgG type antibody, it is preferable that human lymphocyte sufficiently sensitized with an antigen is collected from peripheral blood for use.

When it is difficult to obtain lymphocyte that is sufficiently sensitized with an antigen, antigen sensitization *in vitro* can be also carried out. An antibody of the present invention can be humanized by employing the above-described method and the like, accordingly, the antibody is very useful for administering to a human body.

Further aspect of the present invention is to provide a method for obtaining a novel collectin related molecular species, or novel collectin and/or derived molecules, comprising a step of screening a protein using the antibody set forth above to obtain the novel collectin related molecular species, or novel collectin and/or derived molecules. In this method, a protein having immunoreactivity is screened using the above-described antibody in order to isolate and obtain a novel collectin related molecular species, or novel collectin and/or derived molecules, which may be contained in a variety of body fluids of organisms, tissue cells and the like. In the screening step, the expression screening of cDNA library may be preferably utilized.

In another aspect of the present invention, a method of quantitative determination of a novel collectin and/or derived molecules is provided, comprising measuring an amount of the novel collectin and/or derived molecules contained in a test sample using the antibody set forth above, on the basis of the immunological binding property thereof.

In this method, in order to measure an amount of the novel collectin and/or derived molecules, the above-described antibody may be employed in immunoblotting, immunoprecipitation, ELISA or the like, and in particular, ELISA method that is convenient may be preferably performed. In addition, a sandwich method comprising a step of detecting a sandwich complex generated by reacting a novel collectin and/or derived molecules with an antibody and labeled antibody coupled to, for example, an

insoluble support, otherwise, a competitive method comprising steps of: competitively reacting a labeled novel collectin and/or derived molecules with a novel collectin and/or derived molecules in a test sample with an antibody; and measuring the novel collectin and/or derived molecules in the sample based on the amount of the antigen reacted with the antibody may be utilized to determine the amount of a novel collectin and/or derived molecules.

Upon determination of an amount of a novel collectin and/or derived molecules by the sandwich method, a two steps method, wherein an immunoreaction of an immobilized antibody with a novel collectin and/or derived molecules is allowed first, then unreacted substances are completely removed by washing, followed by addition of a labeled antibody; or a one step method, wherein an immobilized antibody, a labeled antibody, and a novel collectin and/or derived molecules are simultaneously mixed may be performed.

Insoluble support used for such determination may include for example, synthetic resin such as polystyrene, polyethylene, polypropylene, polychlorinated vinyl, polyester, polyacrylate ester, nylon, polyacetal, fluorine contained resin and the like; polysaccharides such as cellulose, agarose and the like; glass; and metal etc. The insoluble support may be in several forms for example, tray-like, spherical, fibrous, cylindrical, discal, vessel-like, cell-like, tubular and the like. The support with the antibody being absorbed may be stored in a cold place, in the presence of an antiseptic agent such as sodium azide.

For immobilization of an antibody, a known chemical binding method or a physical absorption method may be adopted. The chemical binding method may include for example, glutaraldehyde-utilizing method, maleimide method, wherein N-succinimidyl-4-(N-maleimidemethyl) cyclohexane-1-carboxylate, N-succinimidyl-2-maleimideacetate or the like may be used, carbodiimide method, wherein 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride may be used. In addition, maleimidebenzoyl-N-hydroxysuccinimide ester method, N-succinimidyl-3-(2-pyridylthio) propionic acid method, bisdiazotized benzidine method, dipalmityllysine method may be included. Alternatively, a complex which was previously formed by a reaction of a test detected material with an antibody of which epitope is in a different kind, may also be captured after the third antibody to said antibody is immobilized according to the method as mentioned above.

The material to be used for labelling the antibody may be enzyme, fluorescent

material, luminescence material, radioactive material, metal chelate or the like. An enzyme may include for example, peroxidase, alkaline phosphatase,  $\beta$ -D-galactosidase, malate dehydrogenase, staphylococcus nuclease, delta-5-steroid isomerase,  $\alpha$ -glycerolphosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, asparaginase, glucose oxidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholine esterase, and the like; and fluorescent material may include for example, fluorescein isothiocyanate, phycobilin protein, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, orthophthalic aldehyde and the like; luminescence material may include isoluminol, lucigenin, luminol, aromatic acridiniumester, imidazole, acridinium salt and modified ester thereof, luciferin, luciferase, aequorin and the like; and radioactive material may include  $^{125}\text{I}$ ,  $^{127}\text{I}$ ,  $^{131}\text{I}$ ,  $^{14}\text{C}$ ,  $^3\text{H}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$  and the like, but not limited thereto as long as the material can be used in immunological determination methods. Further, low molecular weight hapten such as biotin, dinitrophenyl, pyridoxal or fluorescamine may be conjugated to the antibody. Preferably horseradish peroxidase may be used as a labelling enzyme. This enzyme can react with a lot of substrates, while being readily conjugated to the antibody by a periodic acid method.

When an enzyme is used as a labelling material, a substrate for measuring its activity, and a color-developing agent are employed. When peroxidase is used as an enzyme,  $\text{H}_2\text{O}_2$  may be used as a substrate solution, and 2,2'-azino-di-[3-ethylbenzthiazolin sulfonate] ammonium (ABTS), 5-aminosalicylic acid, orthophenylenediamine, 4-aminoantipyrine, 3,3',5,5'-tetramethylbenzidine or the like may be used as a color-developing agent; when alkaline phosphatase is employed as an enzyme, the substrate may be orthophenylphosphate, paranitrophenylphosphate or the like; alternatively, when  $\beta$ -D-galactosidase is used as an enzyme, the substrate may be fluorescein-di-( $\beta$ -D-galactopyranoside), 4-methyl-umbelliferyl-D-galactopyranoside, or the like.

As a crosslinking agent, N,N'-orthophenylenedimaleimide, 4-(N-maleimidemethyl) cyclohexanoyl N-succinimide ester, 6-maleimidehexanoyl N-succinimide ester, 4,4'-dithiopyridine, or other known crosslinking agents can be utilized. The reaction of such a crosslinking agent with the enzyme and the antibody may be proceeded in accordance with known methods depending upon the properties of the crosslinking agent that was employed.

Additionally, the antibodies to be used may be any fragments of the antibodies for example, Fab', Fab, F(ab')<sub>2</sub> depending on conditions. Furthermore, enzymatically labelled antibody may be prepared by using similar process as any one of polyclonal antibodies and monoclonal antibodies. When the enzymatically labelled antibody that was obtained by using the aforementioned crosslinking agent is purified by any known methods, more sensitive immunological determination system can be achieved. The enzymatically labelled antibody that was purified in such a manner may be mixed with a stabilizer such as thimerosal, glycerol or the like, alternatively, may be lyophilized, and then stored in a cold and dark place.

Test samples for measurement in the above quantitative determination are not limited as far as they are the samples containing a novel collectin and/or derived molecules, or the samples containing a precursor thereof, which may include body fluids such as plasma, serum, blood, urine, tissue fluid, cerebrospinal fluid and the like.

Furthermore, the present invention provides a kit for quantitative determination of a novel collectin and/or derived molecules, comprising the antibody as mentioned above, wherein an amount of the novel collectin and/or derived molecules in a test sample is measured by ELISA method using this antibody. The kit further comprises reagents required for the quantitative determination as described above besides the antibody.

The present invention also provides a method for producing a novel collectin and/or derived molecules, comprising a step of isolating a novel collectin and/or derived molecules using the antibody as above-described. In order to isolate the novel collectin and/or derived molecules in such a method, affinity chromatography and/or immunoprecipitation may be carried out by a column method, a batch method or the like which comprises the steps of: allowing specific interaction between antigen and antibody by contacting a sample with a support coupled to the above-described antibody; washing the support; and eluting the coupled protein intended.

The sample in this method may be for example, the one obtained through preparing from placenta, spleen and the like derived from human, or the one prepared by expression from a polynucleotide encoding the above-described novel collectin of the present invention, however, expression product may be preferably used, since it can be stably supplied as the sample, accordingly, a method comprising such an expression step may be contemplated in the present invention.

Moreover, further aspect contemplated by the present invention is a method for

identifying the presence of a novel collectin and/or derived molecules derived molecules within tissues, wherein immunohistological examination is conducted using the above-described antibody. The immunohistological examination may be for example, techniques such as *in situ* immunohistological staining using a labeled antibody, in which a novel collectin and/or derived molecules may be detected.

Furthermore, in accordance with the present invention, a transgenic non-human animal that is stably introduced with a recombinant gene comprising the above-described polynucleotide according to the present invention into the chromosome, and that can express a novel collectin is provided. Further, provided by the present invention is a transgenic non-human animal wherein introduction of a gene is effected in a non-human animal comprising a homologue of the novel collectin according to the present invention, in a manner to modify (for example, so that the expression is inhibited, promoted or expressed under only limited conditions predetermined) the gene expression of the homologue. In this non-human animal, the expression level of the relevant gene can be artificially modified to be elevated or lowered in comparison to the original gene expression level by eliciting mutations such as deletion, substitution, addition and/or insertion in part of several important sites (enhancer, promoter, intron and the like) that are normally regulating the gene expression. The introduction of the mutations can be carried out by the known methods. (e.g., Lesley, S.A. et al., *Promega Notes Magazine*, 46, 6-10, 1994; Kunkel, T.A. et al., *Methods Enzymol.*, 154, 367-382, 1987; Sayers, J.R. et al., *Biotechniques*, 13, 592-596, 1992; Ito W., et al., *Gene*, 102, 67-70, 1991; Cormack, B., *Current Protocols in Molecular Biology*, 8.5.1-8.5.9, 1987). In this method, the gene may include cDNA, genomic DNA or synthetic DNA. Moreover, the expression of a gene includes both of the steps of transcription and translation.

A transgenic non-human animal according to the present invention may be useful for development of disease model animals for studies on functions, or regulation of expression of the novel collectin and/or the derived molecules, elucidation of the mechanisms involved in the diseases in which the novel collectin and/or derived molecules are expected to participate, and for use in screening and safety test for pharmaceutical products.

The term "transgenic animal" means in its narrow definition, an animal in which an exogenous gene is artificially introduced into germ cells by gene recombination, while in its broader definition, "transgenic animal" means an animal in which an exogenous

gene is stably introduced into chromosomes in an early stage of development of the individual, thus transmission to the descendant thereof can be resulted as an inherited characteristic, including antisense transgenic animals in which a function of a specified gene is suppressed using antisense RNA, animals in which a specific gene is knocked out using embryonic stem cells (ES cell), and animals in which point mutated DNA is introduced.

As used herein, "transgenic animal" may be construed in its broader sense, including all vertebrate animals except human. A transgenic animal of the present invention may be useful for development of disease model animals for studies on functions, or regulation of expression of the novel collectin and/or the derived molecules, elucidation of the mechanisms involved in the diseases related to the cells where those molecules are expressed in human, and for use in screening and safety test for pharmaceutical products.

A method for producing a transgenic animal may include a method in which a gene is directly introduced into a nuclei of the pronucleus stage egg cell using a micropipet under the phase-contrast microscope (micro-injection method, see, US Patent No. 4,873,191), a method in which an embryonic stem cell is used, and the like. Otherwise, a method in which a gene is inserted into a retrovirus vector or a adenovirus vector followed by infection into egg cells; a sperm vector method in which a gene is introduced into an egg cell via a sperm cell, or the like have been developed.

The sperm vector method is a gene recombinant method in which an exogenous gene is attached to a sperm cell or a exogenous gene is incorporated into a sperm cell by electroporation method and the like, followed by introduction by fertilization to an egg cell, resulting in introduction of the exogenous gene (M. Lavitroano *et al*, Cell, 57, 717, 1989). Alternatively, site specific gene recombination *in vivo* by cre/lox P recombinase system of bacteriophage P1, FLP recombinase system of *Saccharomyces cerevisiae*, and the like can be also employed. Moreover, a method of introducing a transgene of the intended protein into non-human animals has been also reported.

A method of producing a transgenic animal by a micro-injection method may be, for example, performed as indicated below.

First, a transgene essentially constituted from a promoter involving in expression regulation, a nucleotide sequence encoding the novel collectin and poly A signal should be prepared. The expression pattern and an amount of expression of a specific molecule

may be affected depending upon the promoter activity, and the produced transgenic animals are different between the strains depending on the number of copies of the introduced transgenes and the introduced sites, therefore, the pattern and the amount of the expression should be confirmed between the strains. Since it is revealed that an amount of expression may vary in connection with an untranslated region as well as splicing, an intron sequence that can be previously spliced in advance of the poly A signal may be also introduced. It is important that a gene used for introduction into a fertilized egg should be a gene having a higher purity as much as possible. The animals for use may include a mouse for collecting fertilized eggs (5-6 weeks), a male mouse for fertilization, a pseudopregnant female mouse, a male mouse received vasoligation, and the like.

In order to efficiently obtain a fertilized egg, ovulation may be induced by gonadotropin and the like. The fertilized egg is recovered, and then a gene placed in an injection pipet is injected into a male pronucleus of the egg in a microinjection method. Animals for bringing back the injected egg to an uterine tube (pseudopregnant female mouse and the like) are prepared, and about 10-15 eggs are transplanted per one animal. Subsequently, extraction of a genomic DNA from the top end of the tail, followed by detection of the transgene by Southern blotting method or PCR method, or positive cloning method wherein a marker gene is inserted which is activated only when a homologous recombination resulted, may be carried out to determine if the transgene is introduced in the mice at birth. Furthermore, in order to confirm the expression of the transgene, a transcription product derived from the transgene is detected by Northern blotting method or RT-PCR method. Alternatively, Western blotting method may be performed with a specific antibody to the protein or a fragment thereof.

Furthermore, according to the present invention, a knockout non-human animal is provided, which is obtained by altering a gene encoding a homologue of the above-mentioned novel collectin according to the present invention in a non-human animal comprising the homologue, in a manner to inhibit the expression of the homologue.

The knockout mouse of the present invention may be treated to deteriorate the function of the gene of the novel collectin and/or derived molecules. The "knockout mouse" means a transgenic mouse in which a given gene is destroyed by homologous recombination technology thus deterioration of its function is resulted. A knockout



mouse can be produced by performing homologous recombination using ES cells, and selecting an embryonic stem cell in which one of the allelic genes was modified/destroyed. A chimera mouse having mixed cells derived from the embryonic stem cell and cells derived from an embryo, may be obtained by, for example, injection of an embryonic stem cell with a gene thereof being manipulated, into blastocyst or morula stage of the fertilized egg. When this chimera mouse (chimera is referred to as a single individual mouse formed with somatic cells based on more than two fertilized eggs) is cross fertilized with a normal mouse, a heterozygote mouse having one of the allelic gene is entirely modified/destroyed can be produced. Furthermore, homozygote mouse can be produced by cross fertilizing the heterozygote mice each other.

The "homologous recombination" means recombination that occurs between two genes having the identical or closely similar nucleotide sequences through a gene recombinant mechanism. A PCR method can be used for selecting the cells with the homologous recombination occurred. A polymerase chain reaction may be carried out using a portion of an inserted gene and a portion of a region expected for the insertion, as primers, consequently, the occurrence of the homologous recombination in the cells can be identified where the amplification product was resulted. In addition, when homologous recombination is caused in a gene that is expressed in an embryonic stem cell, a neomycin resistant gene has been previously ligated to the gene introduced, and after introducing the gene, selection can be readily performed by, for example, leading the cells to be neomycin resistant, or by any another known methods or modified methods thereof (see, Capecchi, *Science*, vol. 244, pp. 1288-1299 (1989), and the like).

The present invention will be described in more detail by the following non-limiting illustrative examples. It is intended that the present invention encompasses all modifications and variations which occur to those skilled in the art upon consideration of the disclosures herein, and in particular those embodiments which are within the broadest proper interpretation of the claims and their requirements.

The Examples demonstrate: the search on EST database (Example 1); preparation of the probe for screening (Example 2); screening of cDNA library derived from human placenta (Example 3); sequencing of nucleotide sequence of the novel collectin (Example 4); genomic Southern analysis of the novel collectin (Example 5); Northern analysis of the novel collectin with various human tissues (Example 6); genomic

Southern analysis of the novel collectin with tissues from various species of animals (Example 7); and genetic study of the novel collectin (Example 8); generation of monoclonal antibodies specific for the novel collectin (Example 9); ELISA method using the anti-mouse anti-human novel collection antibody (Example 10); generation of antibodies with lowered immunogenicity to human (Example 11); construction of an expression vector pNOW1-hCL-P1 of the novel collectin (Example 12); selection of a clone expressing the novel collectin (Example 13); production of a transgenic mouse (Example 14); and production of a knockout mouse (Example 15).

#### Example 1: Search on EST Database

Highly conserved regions between molecules of the known collectins, i.e., human MBP, human SP-A and human SP-D were searched by comparing the amino acid sequences thereof (see Figures 2 and 3, in which amino acid residues which were recognized to be homologous between those proteins were boxed). As a result, it was suggested that the region consisting of 27 amino acids, namely from amino acid 220 to 246 of human MBP sequence (shown in Figure 3, reversed characters), was highly homologous. Therefore, some consensus sequences corresponding to this region were prepared, and searches on EST (Expressed Sequence Tags) database were conducted with such sequences. For this search, the EST database published on October 11, 1996 was employed, which included 676750 sequences.

Consequently, some data comprising highly homologous amino acid sequences with the sequence of the 27 amino acid described above were obtained. Searches on GenBank/EST database with thus obtained data of the amino acid sequences were further conducted, and deduced whether they were derived from known, or unknown genes. Thereby, it was confirmed that data including two highly homologous but unknown nucleotide sequences (registered as: W72977 and R74387) could be obtained when the following amino acid sequence was used as a consensus sequence:  
Glu-Lys-Cys-Val-Glu-Met-Tyr-Thr-Asp-Gly-Lys-Trp-Asn-Asp-Arg-Asn-Cys-Leu-Gln-Ser-Arg-Leu-Ala-Ile-Cys-Glu-Phe (SEQ ID NO: 3). These were respectively derived from placenta and from fetal heart, and clones indicating portions of nucleotide sequences of the novel collectin.

Thereafter, the clone derived from fetal heart (I.M.A.G.E. Consortium Clone ID 34472) was purchased from ATCC (American Type Culture Collection), and utilized as a

screening probe for obtaining a novel collectin.

#### Example 2: Preparation of the Probe for Screening

The nucleotide sequence of insert DNA of the clone described above was determined using primers (Pharmacia, M13 Universal Primer (SEQ ID NO: 7, 5'-fluorescein-cgacgttgtaaacgacggccagt-3') and M13 Reverse Primer (SEQ ID NO: 8, 5'-fluorescein-caggaacagctatgac-3')).

From thus obtained nucleotide sequence, an open reading frame was selected through matching it to the collectin amino acid sequence, and the nucleotide sequence corresponding to the amino acid sequence which could be read from the above open reading frame was picked out. Then, primers for digoxigenin (DIG) labeled cDNA probes (Reverse Primer: caatctgatgagaaggtgatg (SEQ ID NO: 4) and Forward Primer: acgagggtctgatgggacat (SEQ ID NO: 5)) corresponding to the parts of the nucleotide sequences were produced using DNA/RNA Synthesizer (Applied Biosystems, 392A). DIG labeling was achieved using PCR DIG Probe Synthesis Kit (Boehringer Mannheim). The reaction mixture contained: plasmid DNA (clone W72977, 50 ng/ $\mu$ l), 2  $\mu$ l (100 ng); 10 x Buffer 5  $\mu$ l; 25 mM  $MgCl_2$ , 5  $\mu$ l; dNTP (PCR Labeling Mix), 5  $\mu$ l; 20  $\mu$ M Reverse Primer, 2.5  $\mu$ l; 20  $\mu$ M Forward Primer, 5  $\mu$ l;  $H_2O$ , 28  $\mu$ l; Taq Polymerase, 0.5  $\mu$ l. PCR reaction was carried out using Zymoreactor (ATTO Corp.) through 35 cycles of: 1 minute at 92°C, 1 minute at 55°C, and 2 minutes at 72°C.

#### Example 3: Screening of cDNA Library Derived from Human Placenta

First, phage cDNA library derived from human placenta was titrated as follows. Escherichia coli Y1090r, 0.2 ml, which had been cultured at 37°C for 16 hours in mLB medium (LB medium (1 g tryptone, 0.5g yeast extract and 0.5 g NaCl in total volume of 100 ml) containing 10 mM  $MgSO_4$  and 0.2% maltose) and 0.1 ml of cDNA library serially diluted with SM buffer (5.8 g NaCl, 2 g  $MgSO_4 \cdot 7H_2O$ , 2 M Tris-HCl (pH 7.5) 25 ml, and 2% gelatin 5 ml in total volume of 1L) were incubated at 37°C for 15 minutes, then the mixtures were added to 2.5 ml of LB-TOP agarose (0.75% agarose/LB medium) to make homogenous solutions, and plated onto 90 mm  $\phi$  LB Medium Plates (Iwaki Glass), (1.5% agar/LB medium). The added solutions were hardened at room temperature for 15 minutes, then incubated for 5 hours at 42°C. The plaques on each of the plates were counted, and the titer of the phage was calculated. Consequently, the titer calculated to be  $2.1 \times 10^{10}$  pfu/ml. The screening was performed as follows using the

probe prepared in Example 2.

Escherichia coli Y1090r, 0.6 ml, which had been cultured at 37°C for 16 hours in mLB medium, and cDNA library diluted with SM buffer to  $1 \times 10^5$  pfu were incubated at 37°C for 15 minutes, then the mixture was added to 7.5 ml of LB-TOP agarose (0.75% agarose) to make a homogenous solution. The solution was plated onto ten LB square plates of 140 cm<sup>2</sup> (Nissui Seiyaku), hardened at room temperature for 15 minutes, then the plates were incubated for 5 hours at 42°C. After plaque formation of each of the plates was confirmed, the transfer to the nylon membranes was performed. The transfer was performed using Nytran 13N (Schleicher and Schuell Co.). The filters (12.5 cm x 9.0 cm in size) were immersed in distilled water for 10 minutes to be wet, then the excess water was removed on Whatmann 3MM Paper, and the filters were placed on the plates having the plaques formed thereon. After standing for two minutes, the filters were recovered and air-dried for 10 minutes. The phage DNA on the filters was denatured for 2 minutes with 0.2 M NaOH/1.5 M NaCl, followed by neutralization with 0.4 M Tris-HCl (pH 7.6) / 2 x SSC for 2 minutes and washing with 2 x SSC for 2 minutes. Thereafter, the phage DNA was fixed on the membrane by UV irradiation with GS GENE LINKER (BioRad).

Hybridization, and detection of the signals were conducted as follows. The filters were soaked in 2 x SSC, and the excess moisture was removed using Whatmann 3MM Paper. Then, the filters were placed in a hybridization bag and prehybridization at 68°C for one hour in a hybridization solution (5 x SSC, 1% blocking agent, 0.1% N-lauroyl sarcosine and 0.02% SDS) was performed. Subsequently, the hybridization solution was removed from the bag, and the hybridization solution containing DIG labeled cDNA probe at a concentration of 10 ng/ml was added thereto, and hybridization was proceeded at 55°C for 16 hours. After the hybridization was completed, the filters were washed in a solution of 2 x SSC/0.1% SDS for 5 minutes, 2 times; and further washed in a solution of 0.5 x SSC/0.1% SDS for 15 minutes, 2 times. Then, SDS was removed using DIG buffer I (100 mM Tris-HCl, 150 mM NaCl (pH 7.5)) for 1 minute, and the filters were blocked with DIG buffer II (1% blocking agent in DIG buffer I) for 30 minutes. After washing the filters with DIG buffer I for one minute, a solution of alkaline phosphatase labeled anti-DIG antibody (Boehringer Mannheim) which was diluted to 5000-fold in DIG buffer II was added, and the reaction between antigen and antibody were allowed for 30 minutes at room temperature. The filters were then washed twice with DIG buffer I for 15 minutes at room temperature. Through the subsequent

treatment of the filters with DIG buffer III (100 mM Tris-HCl, 100 mM NaCl (pH 9.5), 50 mM MgCl<sub>2</sub>) for 3 minutes, the concentration of Mg<sup>2+</sup> was elevated. A solution of NBT/BCIP (WAKO Chem., Co.) in DIG buffer III was added for color development, accordingly, 10 positive clones were identified.

The plaques corresponding to these clones were excised from the plates and placed in the tubes containing 1 ml of SM buffer. After stirring for 10 minutes, each of the buffer solution was serially diluted with SM buffer, and 0.1 ml of the diluted solution was mixed with 0.2 ml cultures of *Escherichia coli* Y1090r which had been cultured in mLb medium for 16 hours at 37°C. The mixture was incubated for 15 minutes at 37°C, and then added to 2.5 ml of LB-TOP agarose to make a homogenous solution. The solution was plated into ten 90mm φ LB plates, hardened at room temperature for 15 minutes, then the plates were incubated for 5 hours at 42°C. Several plaques were obtained, and the secondary screening was performed essentially in accordance with the procedures of the primary screening.

#### Example 4: Sequencing of the Novel Collectin Nucleotide Sequence

The plaque of the clone that was expected as being appropriate among the positive clones obtained in the above secondary screening was excised from the plates, and was placed into a tube containing distilled water 200 μl followed by stirring for 30 minutes at room temperature, then the tube was centrifuged at 15,000 rpm for 5 minutes, and the supernatant was obtained therefrom.

The insert DNA was amplified by PCR with TaKaRa LA PCR Kit Ver.2 (TAKARA Syuzo, Co.) using the resulting supernatant as a template. PCR reactions contained: the supernatant, 27 μl; 10 x LA PCR Buffer II (Mg<sup>2+</sup> free), 5 μl; 25 mM MgCl<sub>2</sub>, 5 μl; dNTP Mix, 8 μl; 20 μM λgt11 Reverse Primer (SEQ ID NO: 9: 5'-ttgacaccagaccaactgtaatg-3'), 2.5 μl; 20 μM λgt11 Forward Primer (SEQ ID NO: 10: 5'-ggtggcgacgactcctggagcccg-3'), 1 μl; LA Taq polymerase, 0.5 μl; and H<sub>2</sub>O, to make final volume of 50 μl. PCR reaction was performed using Applied Biosystems Gene Amp PCR System 9600, with 30 cycles of: 20 seconds at 98°C, and 5 minutes at 68°C. The PCR product was verified by the electrophoresis on 1% agarose gel, and purified through excising from the gel. For this purification step, Sephaglas BandPrep Kit (Pharmacia) was used.

The excised DNA fragment was incorporated into pCR2.1 vector (Invitrogen, TA Cloning Kit). The recombinant vector was transformed into TOP10F' cell included in the

Invitrogen TA Cloning Kit. The transformants were cultured in LB medium (containing 100 µg/ml ampicillin), and three kinds of plasmids were extracted by alkaline SDS method.

Thus obtained DNA was cleaved with restriction enzymes that were expected to be adequate, and each DNA fragment was incorporated into pUC18 vector followed by transformation into XL1-Blue cell. The transformants were cultured on LB medium (containing 100 µg/ml ampicillin), and the plasmids were extracted by alkaline SDS method. CL-P1-2-1 resulted in a plasmid containing EcoRI-Hind III fragment and Hind III-EcoRI fragment; CL-P1-3-4 resulted in a plasmid containing EcoRI-BamHI fragment, BamHI-SmaI fragment, SmaI-HindIII fragment, KpnI-Sau3AI fragment, Sau3AI-EcoRI fragment, EcoRI-KpnI fragment and EcoRI-SmaI fragment; CL-P1-3-7 resulted in a plasmid containing EcoRI-BamHI fragment, BamHI-SmaI fragment, SmaI-HindIII fragment, KpnI-Sau3AI fragment, Sau3AI-EcoRI fragment, EcoRI-KpnI fragment and KpnI-EcoRI fragment. The primers prepared were: M13 Universal Primer (SEQ ID NO: 5); M13 Reverse Primer (SEQ ID NO: 6) from the Autoread Sequencing Kit; and the following FITC (Pharmacia, Fluore Prime)-labeled primers that were produced using a DNA/RNA synthesizer followed by sequencing of the nucleotide sequences of the entire regions with Autoread Sequencing Kit (Pharmacia) and A.L.F. Autosequencer.

HPP 1: 5'-fluorescein-cgtgaaatgaatggaagtg-3' (SEQ ID NO: 11),

HPP 2: 5'-fluorescein-tttatccattgctgttctc-3' (SEQ ID NO: 12),

HPP 3: 5'-fluorescein-ctggcagtcctcccgaggtccag-3' (SEQ ID NO: 13),

HPP 5: 5'-fluorescein-gctgggtccccccggagagcgt-3' (SEQ ID NO: 14)

The outline of this nucleotide sequencing strategy performed is shown in Figure 4. An ORF of the obtained collectin is illustrated in Figure 4 (a), wherein G-X-Y denotes a collagen-like region. Further, in Figure 4 (b), each of the primer names and positions of the nucleotide sequences that were read from the sequencer (shown as allows), as well as M13 Universal Primer (shown as U) and M13 Reverse Primer (shown as R) are illustrated.

In addition, a nucleotide sequence around the 5'-end region comprising a transcription initiation site was determined using Cap site cDNA.

First PCR was performed with Cap Site cDNA, Human Liver (NIPPON GENE) using TGP1 Primer (5'-tcttcagtttcctaattccc-3'(SEQ ID NO: 16)) that was synthesized with the attached IRC2 Primer (5'-caaggtacgccacgcgtatg-3' (SEQ ID NO: 15)) and 392A

DNA/RNA Synthesizer (Applied Biosystems). The employed reaction mixture contained LA PCR Buffer II ( $Mg^{2+}$  free), 2.5 mM  $MgCl_2$ , each 200  $\mu M$  of dATP, dCTP, dGTP and dTTP (all of which are manufactured by TAKARA Syuzo, Co.), 1  $\mu l$ ; Cap Site cDNA Human Liver; 0.5  $\mu l$  1RC2 Primer (both of which are manufactured by NIPPON GENE), and 0.5  $\mu M$  TGP1 Primer, in total volume of 50  $\mu l$ . PCR was performed using a program comprising 35 cycles of: heat denaturation for 20 seconds at 95°C, annealing for 20 seconds at 60 °C, extension for 20 seconds at 72 °C, with heat denaturation for 5 minutes at 95°C prior to the repeated reaction and final extension for 10 minutes at 72°C. After completing the first PCR, nested PCR was conducted. The reaction was performed using 1  $\mu l$  of the first PCR product as a template, with primers 2RC2 Primer (5'-gtacgccacagegtatgatgc-3' (SEQ ID NO: 17)) attached and synthetic TGP2 Primer (5'-cattcttgacaaactcatag-3' (SEQ ID NO: 18)) that was synthesized similarly to TGP1 Primer, and with the same reaction components and program (except that the cycle number was 25) as in the first PCR. The PCR reaction was performed with TaKaRa PCR Thermal Cycler 480. After thus obtained PCR product was confirmed on agarose gel electrophoresis, the band was excised from the gel, followed by freezing at -80 °C for 10 minutes, centrifuge at 15000 rpm, for 10 minutes, and then the supernatant was purified by ethanol precipitation.

The purified DNA fragment was incorporated into pT7Blue Vector (Novagen), and the vector was transformed into competent XL1-Blue cell. The transformants were cultured on LB medium (containing 100  $\mu g/ml$  ampicillin), and the plasmids were extracted by alkaline SDS method, followed by sequencing of the nucleotide sequence with Autoread Sequencing Kit (Pharmacia) and A.L.F. DNA Sequencer. The employed primers were M13 Universal Primer (SEQ ID NO: 7) and M13 Reverse Primer (SEQ ID NO: 8) attached to AutoRead Sequencing Kit.

As a result, it was confirmed that the cDNA clone of the novel collectin that was obtained in Example 3 contained 2024 bases comprising ORF (open reading frame) of 1026 bases encoding 342 amino acids set out in SEQ ID NO: 2.

Next, the results of homology searches of the DNA and amino acid sequence on GenBank database revealed that the amino acid sequence of the obtained protein is distinct from those of the collectins identified previously and it derives from a novel protein.

In addition, the amino acid sequence of the novel collectin of the present invention

was compared to those of three collectin proteins reported heretofore. The alignment is shown in Figures 5 and 6. Similarly to Figures 2 and 3, homologous amino acid residues were boxed. This alignment suggests that the obtained novel protein shares homology with known collectins and it belongs to the collectin family.

#### Example 5: Genomic Southern Analysis of the Novel Collectin

Genomic Southern analysis was performed in order to clarify whether the novel collectin gene comprising the cDNA sequence verified in Example 4 was a single copy gene or a multi copy gene.

Four µg of genomic DNA (Promega) derived from human blood was digested with any one of the restriction enzymes, (1) EcoRI, (2) XbaI, (3) HindIII, (4) PstI, (5) BglII or (6) BamHI, followed by electrophoresis on 0.8% agarose gel at 100 mA, for 3 hours. After the electrophoresis was completed, the DNA was transferred to a nylon membrane (Nytran 13N) to prepare a membrane for the analysis.

For the transfer step, the electrophoresed gel was first immersed in 100 ml of 0.25 N HCl for 10 minutes, washed three times with distilled water, then immersed twice in 100 ml of a denaturalizing solution (1.5 M NaCl, 0.5 M NaOH) for 15 minutes, and immersed in 100 ml of a neutralizing solution (0.5 M Tris-HCl, 3 M NaCl (pH 6.8)) for 30 minutes so that depurination, denaturation and neutralization were accomplished. Thereafter, the DNA was transferred using Vacuum Blotting System (Toyobo Engineering, VB-30). In this step, the membrane which had been pretreated by immersing in 2 x SSC for 5 minutes and in 20 x SSC for 5 minutes was used, with a pad which had been soaked with 20 x SSC. After the transfer was terminated, fixation of the DNA was performed by UV irradiation.

Hybridization probe employed for the Southern analysis was a DNA probe for a portion of ORF of the cDNA sequence of the novel collectin obtained in Example 4: gaagacaagt cttcacatct tgttttcata aacactagag aggaacagca atggataaaa aaacagatgg tagggagaga gagccactgg atcgccctca cagactcaga g (SEQ ID NO: 21), which was labeled using the above-mentioned PCR DIG Probe Synthesis Kit with primers:

5'-gaagacaagtcttcaactcttg-3' (SEQ ID NO: 19) and

5'-ctctgagtctgtgagcgccgac-3' (SEQ ID NO: 20). Prior to hybridization, the probe was boiled for 10 minutes, and rapidly frozen with dry ice/ethanol for 5 minutes.

First, the membrane that was subjected to the transfer step was immersed in 2 x SSC for 5 minutes, then prehybridization was performed in ExpressHyb Hybridization



Solution (Clonetechn), 10 ml at 65°C for 30 minutes. Subsequently, the above frozen probe was diluted to 10 ng/ml in ExpressHyb Hybridization Solution, and 2 ml of this solution was used for hybridization at 65°C for one hour.

Following hybridization, the membrane was washed by: shaking in 20 ml of 2 x SSC solution at room temperature for 5 minutes two times, then in 20 ml of 0.2 x SSC, 0.1% SDS solution at 65°C for 15 minutes twice. Next, the membrane was washed two times with 50 ml of DIG buffer I (100 mM Tris-HCl, 150 mM NaCl (pH 7.5)) at room temperature for one minute in order to remove SDS, and then blocked in 50 ml of DIG buffer II' (1.5% blocking agent in DIG buffer I) at room temperature for one hour. Thereafter, the membrane was treated with 10 ml of alkaline phosphatase labeled anti-DIG antibody which had been diluted to 5000-fold in DIG buffer I containing 0.2% Tween20 for 30 minutes followed by washing two times by shaking in 50 ml of DIG buffer I which contains 0.2% Tween20 at room temperature for 20 minutes. After soaking the membrane twice in 10 ml of DIG buffer III at room temperature for 3 minutes, it was placed in a hybridization bag, and CSPD (registered trade name, Boehringer Mannheim: chemiluminescence substrate) that had been diluted to 100-fold in DIG buffer III was added thereto so that the solution can spread over the membrane. Subsequently, the membrane was exposed onto Instant Film T612 (Polaroid).

Consequently, it was speculated that the gene of the obtained novel collectin has been a single copy gene, because only one or two signals could be detected from the respective genomic DNA which was digested with each of the restriction enzymes, as shown in the lanes of Figure 7.

#### Example 6: Analysis of Distribution of Human Tissue Specific Expression of the Novel Collectin

In order to examine the expression of the mRNA of the novel collectin of the present invention in various human tissues, analysis was performed by RT-PCR.

RT-PCR was performed using RNA LA PCR Kit (AMV) Ver.1.1 (TAKARA Syuzo, Co.) with each RNA derived from several human tissues ((1) brain, (2) heart, (3) kidney, (4) spleen, (5) liver, (6) small intestine, (7) muscle, (8) testis, (9) placenta, or (10) colon (OriGene Technologies, Inc.)) as a template. First, reverse transcription reaction was conducted in the following reaction mixture. The reaction mixture contained 5 mM MgCl<sub>2</sub>, 1 x RNA PCR Buffer, 1 mM dNTP Mixture, 1 U/μl Rnase inhibitor, 0.25 U/μl reverse transcriptase, 0.125 μM Oligo dT-Adaptor Primer, RNA 1 μg, and was adjusted to

total volume of 20  $\mu$ l with RNase free distilled water. At the same time, a reaction mixture without reverse transcriptase was also prepared for the negative control. The reaction mixture was placed in 0.2 ml tube, and subjected to PCR with TaKaRa PCR Thermal Cycler PERSONAL (TAKARA Syuzo, Co.) through 1 cycle of: 30 minutes at 42°C, 5 minutes at 99°C, and 5 minutes at 5°C. Thus resulted PCR product was subsequently used for LA PCR with the following reaction mixture; 2.5 mM  $MgCl_2$ , 1 x LA PCR Buffer II ( $Mg^{2+}$  free), 2U TaKaRa LA Taq, added with two kinds of 0.2  $\mu$ M primers (RT-PCR Primer U: 5'-gtgcccttgccctgcagaatg-3' (SEQ ID NO: 22) and RT-PCR Primer R: 5'-gcataaccctggggaacattttg-3' (SEQ ID NO: 23) that could amplify a cDNA sequence spanning from neck region to carbohydrate recognition domain of the novel collectin, which was adjusted to total volume of 80  $\mu$ l with sterile distilled water. The reaction product was separated on 1% agarose gel electrophoresis, followed by staining with ethidium bromide solution (0.1  $\mu$ g/ml), verification of the electrophoretic pattern with transilluminator, and identification of the expressed tissue.

Further, in order to compare the expressed amount in each of the tissues, RT-PCR was performed to amplify a part of  $\beta$ -actin with each of the tissues, and the amount of RNA was corrected. The RT-PCR was performed similarly to the above procedure with reverse transcriptase reaction, PCR reaction, and identified using 1% agarose gel electrophoresis as described above. The reaction mixture of the reverse transcription contained 5 mM  $MgCl_2$ , 1 x RNA PCR Buffer, 1 mM dNTP Mixture, 1U/ $\mu$ l RNase inhibitor, 0.25 U/ $\mu$ l reverse transcriptase, 2.5  $\mu$ M random 9 mer, RNA 10 ng, which was adjusted to total volume of 60  $\mu$ l with RNase free distilled water. PCR was performed through 1 cycle of: 10 minutes at 30°C, 15 minutes at 42°C, 5 minutes at 99°C and 5 minutes at 5°C. Thus resulted PCR product was subsequently used for PCR with the following reaction mixture; 1 x LA PCR Buffer II ( $Mg^{2+}$  free), 2U TaKaRa LA Taq, 0.25  $\mu$ M human  $\beta$ -actin sense primer 5'-caagagatggccacggctgt-3'(SEQ ID NO: 24), 0.25  $\mu$ M human  $\beta$ -actin antisense primer 5'-tcctctgcatcctgtggca-3'(SEQ ID NO: 25), which was adjusted to total volume of 40  $\mu$ l with sterile distilled water. PCR was performed through 30 cycles of: 15 seconds at 94°C, and 30 seconds at 68°C.

The results are shown in Figure 8, suggesting that mRNA of the novel collectin of the present invention has been expressed in placenta (lane 9), spleen (lane 4), and kidney (lane 3). Extremely high expression in placenta is clearly suggested.

#### Example 7: Genomic Southern Analysis of the Novel Collectin

from Various Animals

In order to elucidate conservation of the collectin gene of the present invention in the other species of animals, analysis by genomic Southern hybridization was performed.

Hybridization probe employed for this analysis was the DIG labeled DNA probe corresponding to the cDNA sequence of ORF of the novel collectin as described above, which was labeled using the above-described PCR DIG Probe Synthesis Kit (Boehringer Mannheim). The employed membrane was prepared by treatment of each 5 µg of genomic DNAs of (1) human (Promega), (2) monkey (Clontech), (3) rat (Promega), (4) mouse (Promega), (5) dog (Clontech), (6) cow (Promega), (7) rabbit (Clontech), and (8) chicken (Promega) with restriction enzyme EcoRI, electrophoresis on agarose gel, transfer to Nytran 13N membrane, and fixation by UV irradiation.

Using the probe and membrane, hybridization was carried out according to the following procedures. First, the membrane was immersed in 2 x SSC for 5 minutes, then prehybridization was performed in 10 ml of ExpressHyb Hybridization Solution at 65°C for 30 minutes. Subsequently, the probe that had been frozen as described above was diluted in the ExpressHyb Hybridization Solution to be 10 ng/ml, and 2 ml of thus diluted probe solution was used for hybridization at 65°C for one hour.

Following hybridization, the membrane was washed by: shaking in 20 ml of 2 x SSC, 0.1% SDS at room temperature for 5 minutes two times, and then shaking in 20 ml of 0.2 x SSC, 0.1% SDS at 68°C for 15 minutes two times. Next, the membrane was washed two times with DIG buffer I at room temperature for one minute in order to remove SDS, and was blocked in 50 ml of DIG buffer II' at room temperature for one hour. Thereafter, the membrane was treated with 10 ml of alkaline phosphatase labeled anti-DIG antibody which had been diluted to 5000-fold in DIG buffer I which contains 0.2% Tween20 for 30 minutes followed by washing two times with shaking in 50 ml of DIG buffer I which contains 0.2% Tween20 at room temperature for 20 minutes. After soaking the membrane twice in 10 ml of DIG buffer III at room temperature for 3 minutes, it was placed in a hybridization bag, and CSPD that was diluted to 100-fold in DIG buffer III was added thereto so that the solution can spread over the membrane. Subsequently, the membrane was exposed to Instant Film T612.

The result of this analysis is shown in Figure 9, wherein signals of DNA can be found in all lanes except for the lane 8 which was loaded with DNA from chicken. Therefore, it was demonstrated that the novel collectin gene of the present invention has

been conserved between the mammalian species.

Example 8: Genetic Analysis of the Novel Collectin

To elucidate the genetic positional relevance of the present collectin between the known collectins, analysis was performed based on the DNA sequence of the novel collectin as obtained, and a phylogenetic tree was created.

The collectins selected as subjects for analysis were several kinds of proteins belonging to the collectin family shown in Figure 10 (in Figure 10, CL-L1 designates a collectin derived from human liver, which was recently isolated by the present inventor (see, Japanese Patent Application No. Hei 10-11281)). Each of the amino acid sequence was retrieved from GenBank database, and then, multiple alignments were produced by clustalw method using the regions containing lectin domains from the obtained data to create the phylogenetic tree using N-J (neighbor-joining) method with Phylip Version 3.57c package program.

Consequently, as shown in Figure 10, although SP-D, bovine collectin-43 and bovine conglutinin have constituted single cluster, additionally MBP and SP-A have respectively constituted separate clusters, while the collectin gene of the present invention has not belonged to any of these clusters similarly to CL-L1. Furthermore, it was speculated that the collectin of the present invention may constitute a distinct cluster which is genetically diverse from those of the collectins reported heretofore including CL-L1.

Example 9: Generation of Monoclonal Antibodies to the Novel Collectin

A solution of 100 µg of the novel collectin dissolved in 100 µl of PBS (-) (PBS, free from calcium ion and magnesium ion), and 100 µl of Freund's complete adjuvant are mixed to prepare an emulsion, and the emulsion is intraperitoneally injected into two BALB/c mice (male, 8 weeks). After three weeks, the second immunization is similarly conducted. However, Freund's incomplete adjuvant is employed instead of Freund's complete adjuvant this time. After additional two weeks, serum is recovered and the antibody titer thereof is measured. A mouse having the highest antibody titer is selected, and after one week passed, the final immunization is conducted similarly to the second immunization. After 5 days from the final immunization, the spleen is taken, and a cell suspension liquid is prepared. The cell fusion is performed by mixing  $2 \times 10^7$  NS-1 myeloma cells and  $1 \times 10^8$  spleen cells prepared. After centrifuging the mixture at 1,600 rpm for 6 minutes at room temperature, the supernatant is removed, then the remaining

cells are loosen, and 1 ml of polyethylene glycol (PEG) is added thereto for a time period of one minute, followed by stirring for one minute at room temperature. Subsequently, 1 ml of culture medium (Iscove's medium, containing 15% FCS, 2 mM L-glutamine solution, 0.05 mM 2-mercaptoethanol) previously incubated at 37°C is added dropwise for a time period of one minute with gentle mixing. Following a repeat of this procedure, then a similar procedure is carried out with 8 ml of the same for a time period of 3 minutes. Subsequently, PEG is removed by centrifuging at 1,000 rpm for 5 minutes at room temperature. The pellet is loosen, to which 100 ml of  $5 \times 10^6$  cells/ml of thymocyte suspension liquid (for use as feeder cells enhancing hybridoma formation rate, which is suspended in hybridoma growth medium including HAT) is added, to prepare a suspension liquid. This suspension liquid is plated into a 96 well-plate, each 0.1 ml per well, then cultured at 37°C in 5% CO<sub>2</sub> in a CO<sub>2</sub> incubator.

The selection of hybridoma is carried out as follows. After one day of the initiation of the culture, 150 µl of HAT medium (hybridoma growth medium containing HAT) is added, followed by culture for the subsequent two days. After two days, the medium is replaced by removing 150 µl of the medium, and adding 150 µl of HAT medium thereto, on the following 3 and 7 days of culture, the medium is similarly replaced. After four days, 100 µl of culture supernatant is collected, then the screening is carried out through measuring the antibody titer for the novel collectin. Next, the positive cells for the antibody are transferred from the 96 well plate to a 24-well plate, using a Pasteur pipet. On this occasion, 0.5 ml of HT medium (hybridoma growth medium containing hypoxanthine and thymidine) has been previously added to each well. After culturing 3 days, a portion of the medium is collected, and its antibody titer is again measured, consequently, the cells capable of being determined as antibody-positive are cloned and frozen for storage. For cloning, the positive cells are subjected to limiting dilution in the thymocyte suspension liquid to adjust to 1 cell/ml, each of 0.2 ml is plated into a 96 well-multi plate. After culturing 10 days, the antibody titer of the well that forms single colony per well is measured, and the two colonies indicating higher antibody titers are selected. These two clones are subjected to recloning. The cloning method is carried out similarly to the method of the first cloning. Subsequently, the extensive culture of the antibody-positive cell is conducted. First, after transferring the cell to a 24 well-multi plate, the cell is cultured for 3 days, followed by transfer to a 25 cm<sup>2</sup> flask, further culturing 3 days, and again transfer to 225 cm<sup>2</sup>, and culturing 3 days, accordingly,

a monoclonal antibody is prepared by recovering the culture supernatant.

Moreover, for generating a monoclonal antibody from ascites fluid, the cloned hybridoma is adjusted to  $1 \times 10^7$  cells/ml, and then 1 ml per mouse is injected intraperitoneally. After one week, the mouse with enlarged abdomen is anesthetized with ether, and the ascites fluid is collected. The ascites fluid is transferred to a coagulation promoting type centrifugation tube added with serum separation agent, followed by allowing to stand for 30 minutes at room temperature, and centrifugation at 3,000 rpm for 5 minutes. Ascites fluid from upper portion is recovered, and then the fluid is further purified by ammonium sulfate fractionation to obtain an antibody.

Example 10: ELISA Method Using Anti-mouse Anti-human Novel Collectin Antibody

Anti-mouse anti-human novel collectin antibody is diluted in coating buffer (50 mM carbonate - bicarbonate buffer, pH 9.6) to 10 µg/ml, and the antibody liquid is dispensed 100 µl per well in a 96-well ELISA plate. Subsequently, the plate is incubated at 4°C overnight. The wells were washed three times with 300 µl of a wash liquid (TBS/T liquid (25 mM Tris-HCl, 0.14 M NaCl, 5 mM KCl, 0.05% Tween 20, pH 7.4)). Hereinafter, washing may be similarly performed. Three hundred µl of 5% skim milk/TBS/T liquid is respectively dispensed to the wells, and allowed to stand at 37°C for one hour. After washing, 100 µl of a test sample and a novel collectin standard are dispensed thereto, and the plate is allowed to stand at 37°C for one hour. On this occasion, the test sample is properly diluted, and two-fold serial dilution is employed up to the highest concentration of 0.02 µg/ml for the standard. After washing, 100 µl of 1 µg/ml biotinized anti-rabbit anti-human novel collectin antibody is dispensed thereto, and the plate is allowed to stand at 37°C for 30 minutes. After further washes, 100 µl of biotin streptavidin solution is dispensed thereto, and the plate is allowed to stand at 37°C for 30 minutes. After additional washes, 100 µl of 3, 3', 5, 5'-tetramethylbenzidine solution is dispensed, and the plate is allowed to stand for 30 minutes at room temperature, followed by addition of 1N phosphoric acid 100 µl, termination of the reaction, and measurement of the absorbance at 450 nm of the wavelength.

A calibration curve with the standard is produced using this method, and the quantitative determination of the novel collectin is accomplished.

Example 11: Generation of Antibodies with Lowered Immunogenicity to Human

First, mRNA is prepared from the mouse hybridoma obtained in the method described in Example 9, a cDNA library with a phage vector is prepared, and then cDNA cloning is performed. A mouse antibody cDNA is cloned using cDNA of a constant region as a probe, followed by analysis of the inserted cDNA with restriction enzymes, and then the nucleotide sequence of the antibody cDNA is sequenced.

Next, a chimeric antibody is expressed, and the presence or absence of a mutation in the cloned antibody gene is confirmed from the antibody binding activity, while it may be used as a positive control for studying the binding activity of the expressed humanized antibody. When the chimeric antibody is expressed, a cassette type expression vector in which a constant region gene of human antibody H chain and L chain is cloned on single vector may be employed. Binding activity of the chimeric antibody is determined, resulting in confirmation that the gene is undoubtedly the cloned mouse antibody gene, then a humanized antibody gene is constructed. In the case of preparing a humanized antibody, a gene must be designed for construction, as is different from the case of chimeric antibody generation method. On the basis of the determined nucleotide sequence of the mouse antibody variable region gene, an amino acid sequence of a human antibody variable region that is highly homologous is searched by utilizing a database. The sequences having 70-80% of homologies other than CDR sequence are found, thereafter a mouse antibody CDR sequence is grafted to the region in which CDR sequence is removed from thus selected human antibody variable region (framework region), accordingly, molecular modeling is carried out by a protein structure analysis software. A few amino acids of the respective H chain and L chain in a sequence of the human framework region are returned back to those of the mouse type so that the configurations of 6 CDRs in total on the H chain and L chain that bind to an antigen through modeling become closer to those of the original mouse monoclonal antibody in respect to the three dimensional structure. In such a case, returning the less number of the amino acids to the mouse type is more desirable, because the closer antibody to the human antibody can be resulted. According to this molecule modeling, more than 10 kinds of versions of humanized antibody genes are designed, expressed and activities thereof should be compared, because the probability that the humanized antibody started from one model can reproduce an activity of 100%, equivalent to that of the mouse monoclonal antibody would be low.

After the gene design is completed, the construction of the gene is actually

performed. A cassette type expression vector including a constant region gene of a humanized antibody H, L chains is cloned, then a transient expression is carried out with COS cells and the like, thus the antibody activity of the culture medium supernatant is determined by ELISA method. Several kinds having strong activities are selected, and the stable expression cells are established by employing the respective expression vectors for stable expression and host cells such as COS cells, to prepare the purified antibody from the culture medium supernatant.

Example 12: Construction of an Expression Vector pNOW1-hCL-P1 of the Novel Collectin

First, a sequence spanning from the initiation codon to termination codon of the novel collectins set out in SEQ ID NO: 1 is amplified using a primer consisted of the nucleotide sequence: aaggaaaaaa gcggccgcat gcaacaagat ttgatgagg (SEQ ID NO: 26), and a primer consisted of the nucleotide sequence gctctagatt ataatgcaga tgacagtac (SEQ ID NO: 27) with Zymoreactor (ATTO Corp.).

The cDNA of the novel collectin obtained is digested with restriction enzymes NotI and XbaI, yielding a cDNA portion corresponding to 670-1698 bp of cDNA of the novel collectin, which may be an insert.

Next, the expression vector pNOW/CMV-A (see, Japanese Unexamined Patent Publication No. H10-179169 (1998)) is digested with restriction enzymes NotI and XbaI, then the above insert is inserted at downstream of cytomegalovirus promoter (pCMV), namely, between P<sub>CMV</sub> (cytomegalovirus major antigen early promoter) and BGP poly A (bovine growth hormone polyadenylation signal), using a DNA ligation kit (Takara Shuzo Co., Ltd.). The expression vector thus obtained is designated as plasmid pNOW1-hCL-P1 (hCL-P1: a nucleotide sequence encoding the novel collectin), and a schematic drawing of its structure is shown in Figure 11.

Example 13: Selection of a Clone Expressing the Novel Collectin

(1) Introduction of the expression vector pNOW1-hCL-P1 into dihydrofolate reductase deficient Chinese hamster ovary (CHO) cells (dhfr<sup>-</sup>)

Iscoe's Modified Dulbecco's Medium (IMDM, GIBCO) that is added with 10% of fetal calf serum (FCS, GIBCO) (free from hypoxanthine and thymidine) is prepared, to which DHFR gene deficient DG44 CHO cell strain (dhfr<sup>-</sup>) is mixed to yield  $1 \times 10^5$  cells/ml, which is seeded into a dish having 60 mm diameter, and cultured at 37°C for 24 hours under a condition of 5% carbon dioxide (CO<sub>2</sub>). The culture medium supernatant is



discarded, and in turn, IMDM containing 100  $\mu$ l solution separately and previously obtained by mixing 5  $\mu$ g of DNA (expression vector pNOW1-hCL-P1) with lipofectin solution (DOTAP: Liposomal Transfection Reagent; Boeringer Mannheim) with 10% FCS is added to be 6 ml, further, hypoxanthine (final concentration of 10 nM) (GIBCO) and thymidine (final concentration of 100 nM) (GIBCO) are added thereto, followed by culturing for 16 hours, to introduce the expression vector pNOW1-hCL-P1 into the host dhfr<sup>-</sup> CHO cells of. Thereafter, the culture medium supernatant is discarded, followed by addition of 6 ml of IMDM added with 10% FCS, hypoxanthine, and thymidine, and further culture for 24 hours.

#### (2) Acquisition of neomycin (G418) resistant CHO cells

After the cells in which the expression vector pNOW1-hCL-P1 is incorporated are cultured for 24 hours, which are trypsinized and recovered from the dish, the number of the cells are counted, and thereafter the mixture in which those cells are suspended in IMDM containing neomycin (G418) at a concentration of 400  $\mu$ g/ml with 10% FCS is seeded (dispensed) into ten 96-well micro plates at an amount of 0.1 ml/well. The plates are cultured at 37°C under a condition of 5% carbon dioxide (CO<sub>2</sub>), and after two weeks, the surviving cells are acquired as G418 resistant cells (clone).

These G418 resistant clones are identified for hCL-P1 producibility. Several clones are selected among the clones in which the production of hCL-P1 is confirmed, and then each of the selected clones is seeded into a culture flask of 25 cm<sup>2</sup>. The culture is carried out until the cells become confluent (approximately  $3 \times 10^6$  cells/25 cm<sup>2</sup> culture flask). The culture medium supernatant from each culture flask is discarded, then 2 ml of the IMDM containing 10% FCS identical to the composition described above is added thereto, followed by culture for four days, and recovering the culture medium supernatant. The produced amount of hCL-P1 (rhCL-P1: recombinant human novel collectin) in the recovered culture medium supernatant is measured. It should be noted that the produced amount of hCLP1 may be quantitatively determined according to the method of Suzuki *et al.* (Y. Suzuki, *et al.*, "Characterization of Recombinant Bovine Conglutinin Expressed in a Mammalian Cell", *Biochem. Biophys. Res. Commun.*, **238**:856-863 (1997)) using an anti-rabbit polyclonal antibody to hCL-P1, carbohydrate recognition domain (CRD) and neck region of collectins (expressed in *E. coli* using a similar method), that is expressed in *E. coli* using the method of Suzuki, *et al.*, *supra*, as well as hCL-P1 (quantitative reference) as a control.

### (3) Acquisition of methotrexate resistant CHO cells

Following further passage culture and stabilization of the hCL-P1 producing clone obtained in the above-described Example 13 (2), low concentration of methotrexate (MTX) is added to the medium, and gene amplification is effected.

First, the selected each cell clone is mixed in IMDM containing 5 nM MTX, 400 µg/ml of G418 with 10% FCS previously dialyzed (JRH Bioscience), is seeded (dispensed) into ten 96-well micro plates at 0.1 ml/well. The cells are cultured at 37°C under a condition of 5% carbon dioxide (CO<sub>2</sub>), and after two weeks, the surviving cells are acquired as 5 nM MTX resistant cells (clone).

These 5 nM MTX resistant clones are identified for hCL-P1 producibility. Several clones are optionally selected among the clones in which the production of hCL-P1 is confirmed, and then each of the selected clones is seeded into a culture flask of 25 cm<sup>2</sup> followed by two weeks culture until the cells become confluent. The culture medium supernatant is discarded, then 2 ml of the IMDM containing 10% FCS identical to the composition described above is added thereto, followed by culture for four days, recovering the culture medium supernatant, and then the production level of hCL-P1 is measured. It should be noted that the quantitative determination of the produced amount of hCLP1 may be performed according to the ELISA method described in Example 10.

### Example 14: Production of a Transgenic Mouse

Collectins are believed to have important roles in basic immunity. Additionally, since the novel collectin of the present invention is conceived to form a genetically independent cluster from the conventional collectins, a transgenic non-human animal of this novel collectin is produced in order to obtain new knowledge on immunity and to provide important tools for elucidating the mechanisms of action of the novel collectin.

A plasmid is constructed, which is capable of expressing the novel collectin derived from human of the present invention in a mouse. First, a 2.3 kb fragment is excised by digestion with Sal I/Pst I from a mammalian animal expression vector comprising a chicken β-actin promoter and a cytomegalovirus enhancer at upstream of the β-actin promoter (Niwa, H. *et al.*, *Gene*, **108**:193-200, 1991), which is inserted into Sal I/Pst I site of a cloning vector pBluescript (Stratagene), resulting in construction of pBsCAG-2 vector. In this 2.3 kb fragment, the above described enhancer/ promoter and a portion of rabbit β-globin gene (the second intron, the third exon, and 3'-noncoding region) are contained. The cDNA of hCL-P1 (SEQ ID NO: 1, 670-1698) is inserted into

an Eco RI site of the above- described third exon, and a plasmid p $\beta$ A/hCL-P1 for use in expression in a transgenic mouse is constructed.

Next, a large amount of plasmid p $\beta$ A/hCL-P1 is prepared by an alkaline-SDS method, and the plasmid is purified by CsCl equilibrium density-gradient ultracentrifugation method. A gene is isolated by digestion of p $\beta$ A/hCL-P1 with Sal I/Bam HI, thus the portion of the vector is excised. Subsequently, the gene is separated from the vector DNA fragment by agarose gel electrophoresis, and the introduced DNA fragment is excised from the gel, followed by purification of the DNA fragment using GeneClean II Kit. After ethanol precipitation, the DNA fragment is dissolved in DNA solution for injection (10 mM Tris-HCl (pH 7.5), 0.25 mM EDTA), followed by centrifugation at 15,000 rpm for 5 minutes and the supernatant is recovered. DNA concentration is calculated through measuring absorbance using spectrophotometer, and after adjusting the concentration to 500 DNA molecules/p1, the solution is dispensed to 50 ml, and stored at -20°C until the DNA is introduced into the first phase embryo of a mouse.

Next, fertilized mouse eggs for injecting DNA is prepared. First, in order to induce superovulation in female mouse for use of collecting fertilized eggs, 5 IU/body of pregnant horse serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) are administered intraperitoneally at intervals of 48 hours, and the female mouse is subjected to cohabitation with a male mouse. Abdominal cavity of the female mouse is opened, of which vaginal plug is formed, then the uterine tube is eviscerate, washed with BWB-Hepes medium (containing NaCl 102.2 mM, KCl 4.78 mM,  $\text{KH}_2\text{PO}_4$  1.19 mM,  $\text{CaCl}_2$  1.17 mM,  $\text{MgSO}_4$  1.19 mM,  $\text{NaHCO}_3$  4.76 mM, Hepes 15 mM, sodium lactate 25 mM, sodium pyruvate 0.25 mM, glucose 5.56 mM, BSA 4 mg/mL, EDTA 0.1 mM and phenol red 0.00025%), thereafter, placed in BWB-Hepes medium including 300 mg/ml of hyaluronidase. Ampulla of the uterine tube is cleaved by a watch marker's tweezer under a stereoscopic microscope, then the fertilized egg is dislodged into the medium, picked up by a glass pipet, and washed five times in BWB-Hepes medium. Thereafter, the fertilized egg is placed into a drop of BWB medium covered by mineral oil, and cultured at 37°C under a condition of 5%  $\text{CO}_2$  in an incubator.

Subsequently, the DNA is injected into the fertilized egg pronucleus. In a 60 mm petri dish, a drop of BWB-Hepes medium and DNA solution (500 DNA molecules/p1) is generated, covered with mineral oil. Using injectors respectively equipped with an

injection pipet and a holding pipet, the fertilized egg is entered into the drop over the BWW-Hepes medium. The DNA solution is aspirated in the injection pipet, and the injector is adjusted to be slightly positive pressure, so that the DNA solution is allowed to flow continuously from the tip. The fertilized egg is sucked by the holding pipet so that the male pronucleus is positioned to the injection pipet side, and then the magnification of the objective lens is changed to 40 fold followed by focusing to the pronucleus, the position of the tip of the injection pipet is adjusted to the same plane with the pronucleus.

Subsequently, the tip of the injection pipet is penetrated into the pronucleus, and the DNA solution is injected. After swelling of the nucleus is confirmed, the pipet is quickly drawn, then the injected egg is moved to the lower drop, and manipulated so that mixing with the untreated eggs is avoided. About 30 eggs are subjected to injection once, subsequently, moved to a drop of the BWW medium, and cultured at 37°C under a condition of 5% CO<sub>2</sub> in an incubator.

After culturing, the transplantation of the fertilized eggs to uterine tube is carried out. Nembutal injection liquid is diluted to 10-fold by a diluent (to a final concentration of 5 mg/ml), and 0.01 ml of the solution is administered intraperitoneally per 1 kg of weight, to anesthetize a pseudopregnant mouse. About 30 eggs are injected into a uterine opening of the uterine tube, using a transfer pipet which has been previously sucked the eggs therein along with the medium as little as possible (0.5-1.0 ml). The ovary and uterine tube are returned within the body, and the opening is sutured. Subsequently, the breeding is continued, and approximately 19 days later, an infant is born.

Screening of the transgenic mouse is carried out by dot blot hybridization as follows. The obtained infant is weaned at four weeks after birth, of which tail is cut at about 1 cm from the top under anesthesia, and the piece is placed in an Eppendorf tube of 1.5 ml. STE buffer (50 mM Tris-HCl, 100 mM EDTA (pH 7.5), 0.5% (w/v) SDS) containing 0.5 mg/ml of proteinase K is added at 0.5 ml to the tube, which is subjected to shaking at 55°C in an incubator overnight. One hundred µl of the tail portion solution is transferred to a new tube, then TE saturated phenol 200 µl and TE buffer 110 µl are added thereto, followed by subjecting to the vortex mixer for 5 minutes, and the mixture was centrifuged at 15,000 rpm (20,000 x g) for 5 minutes at room temperature. Two hundred µl of thus resulted aqueous layer is transferred to a new tube, and 200 µl of phenol/chloroform is added thereto, followed by subjecting to the vortex mixer for 5

minutes, and the mixture is centrifuged at 15,000 rpm (20,000 x g) for 5 minutes at room temperature. The supernatant is transferred to a new tube, 20 µl of 3M sodium acetate and 500 µl of ethanol are added thereto, then the mixture is sufficiently shaken for mixing until the filamentous precipitation of DNA can be visually observed, thereafter, subjected to centrifugation at 15,000 rpm (20,000 x g) at 4°C for 5 minutes. After removing the supernatant followed by washes with 70% ethanol, and drying for 5 minutes, the DNA precipitate is dissolved in 100 µl of 2M NaCl, 0.1 M NaOH solution. Following heating for 5 minutes in a boiling water bath, the DNA is rapidly quenched in ice-cold water, thus denaturation of the DNA is resulted. After a 96-well manifold is set, the DNA is spotted on a nylon membrane, then the membrane is air-dried, and heated at 80°C for 2 hours. Hybridization is performed by a conventional method using as a probe a portion without a sequence derived from mouse among the introduced DNA. As a result, the individual in which the signal can be confirmed is identified as transgenic mouse.

When the manipulation of gene injection is thus performed into the fertilized eggs, about 90% of the fertilized eggs keep surviving. All of these surviving eggs are transplanted into recipients at approximately 25 eggs per one animal so that the recipients become pregnant, and about 19th day of the pregnancy, the born infant can be obtained. Genetic analysis of these infant mice is performed, and the existence of the introduced gene is confirmed. Furthermore, F1 mouse is obtained from these transgenic mice, and transmission rate of the transgene is also examined.

#### Example 15: Production of a knockout mouse

First, the construction of DNA for targeting is conducted by incorporating a neomycin resistant gene, which is a selectable marker gene, into an exon portion of a part of the objective gene coding a mouse homologue of a novel collectin of the present invention in ES cell derived from C57BL/6J strain mouse. Next, electroporation of the DNA to ES cells is carried out as follows. The medium of ES cells at a logarithmic growth phase (one 90 mm petri dish) is changed to a fresh ES medium (80% DMEM medium containing 20% FCS, 1 mM pyruvic acid solution, 0.1 mM non-essential amino acid solution and 1 mM 2-mercaptoethanol), followed by additional culture for 4 hours, then after removing the medium and sufficient washes with PBS (-), the cells are treated with 0.1% trypsin for 3 minutes. The cells are added with 1 ml of the medium, then dispersed to single cells, centrifuged at 600 rpm at 4°C for 5 minutes, then the after discarding the supernatant, the cells are suspended in 5 ml of PBS (-), and the number of

the cells are counted. Again, the cells are centrifuged at 600 rpm at 4°C for 5 minutes, and the supernatant is discarded, thereafter the cells are suspended in PBS (-) to be  $1.1 \times 10^7$  cells/ml. From the suspension of  $1.1 \times 10^7$  cells/ml, 0.9 ml is transferred to a cuvette, which is added with 1 µg/µl of DNA solution (for targeting) 25 µl (25 µg), and allowed to stand at room temperature for 5 minutes. The electroporation is carried out using a gene pulser under a condition of 500 µF and 230 V, followed by standing for 5 minutes, the cells are suspended in ES medium (-G418), and seeded each 1/4 portion on four petri dishes (90 mm) that has been plated with feeder cells. After the electroporation is completed, followed by culture for 24 hours, the medium is changed to ES medium containing 150 µg/ml G418 (active type), and the medium is changed on the following everyday. Seven days later, the cloning is carried out as follows.

The medium is removed from the 90 mm petri dish in which the colony appeared, and 10 ml of PBS (-) is dispensed. Colonies in good shapes observed under a stereoscopic microscope are scraped with a tip respectively, sucked, and each of the colonies is transferred to a 96-well plate in which 70 µl of 0.1% trypsin solution has been previously placed. The plate is allowed to stand at 37°C for 4-5 minutes as it is, followed by trypsinization, and after pipetting is sufficiently conducted using a 8 Channel Pipetman, the cells are seeded into four wells of a 24-well plate (with feeder cells adsorbed) in which 0.8 ml of ES medium containing 75 µg/ml G418 has been previously placed. After the culture for 24 hours, the medium is changed to ES medium containing 75 µg/ml G418. Three days later, 100 µl of 0.1% trypsin is added to the clone at a logarithmic growth phase, and after 100 µl of ES medium is added thereto followed by pipetting, the clone is transferred to a round bottom 96-well plate. When all of the 48 clones are treated, 20 µl is taken for culture, while 180 µl of the remainder is used for PCR.

After the clone for PCR in the 96-well plate is centrifuged at 1,200 rpm, at 4°C for 5 minutes, the medium is removed, and the cells are washed three times with PBS (-). One hundred µl of the dissolving solution (100 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.2% (w/v) SDS, 100 mM NaCl, 100 µg/ml proteinase K) is added to the clone using a 8 Channel Pipetman, thus proteinase K-treatment is carried out at 55°C for 1 hour. The plate is sealed with a tape adhered around the plate, and further incubated at 95°C for 15 minutes for complete inactivation of proteinase K. The plate is cooled at 4°C for 5

minutes, 2-3  $\mu$ l of the solution is collected as a sample for PCR, and used for the first screening (the remaining solution is stored at  $-20^{\circ}\text{C}$ ). The sample may be a group consisting of 4 clones, and PCR is performed using a sense primer (atgcacaagattgatgagg (SEQ ID NO: 28) and an antisense primer (cctaccggtagaattgacc (SEQ ID NO: 29), followed by identification of the positive groups through determination of the objective gene by the electrophoresis method. Among the remaining samples that have been conserved at  $-20^{\circ}\text{C}$ , each clone belonging to the positive groups are similarly subjected to PCR, and the positive clones are identified. Next, the screening for clones for use in culture is performed. Twenty  $\mu$ l of the clone for the culture is seeded into four wells/line of a 24-well plate (with feeder cells adsorbed), and when all wells are filled, the plate is placed in a  $\text{CO}_2$  incubator and cultured, then at a final stage, one clone is divided into three wells and successively seeded into a 6 wells-plate (with feeder cells adsorbed).

Generation of a germ line chimera mouse is performed by a sandwich method where an ES cell mass in which a gene is introduced is sandwiched between two pieces of 2.5 days old embryos (8 cells-phase embryo) of a mouse, which are prepared as described below.

The preparation of 2.5 days old embryo is conducted as follows. Five IU of PMSG (pregnant mare serum gonadotropin) is administered intraperitoneally into a C57BL 6J strain female mouse for use of collecting eggs, on 6 days before the adhesion with the 2.5 days ES cells is conducted. On 4 days therebefore, 5 IU of hCG (human chorionic gonadotropin) is additionally administered intraperitoneally, and the mouse is lead to mating with a male. On 3 days before, mating is confirmed by vaginal plug formation, and a female mouse for pseudopregnant being naturally estrous is lead to mating with a male mouse received vasoligation. The mating is confirmed by vaginal plug formation, thus the female mouse is identified as a pseudopregnant mouse. On the day of adhesion, the female mouse for collecting eggs is subjected to euthanasia by dislocation of cervical vertebrae, and then uterine tube and uterus are removed. The uterine tube and uterus are refluxed with BWB-Hepes medium, 2.5 days embryos (8 cells-phase embryos, or morula embryos) are collected, transferred to a drop of BWB medium, and cultured in a  $\text{CO}_2$  incubator (5%  $\text{CO}_2$ ,  $37^{\circ}\text{C}$ ). The eggs are collected, and the 2.5 days embryos are transferred to a drop of an acidic Tyrode's solution (200  $\mu$ l), allowed to stand for 1-2 minutes, thereafter, when clear zone is dissolved, the eggs are

immediately washed six times with BWV-Hepes medium and is used for adhesion with the ES cells.

The preparation of ES cells is conducted as follows. On the day of adhesion, ES cells cultured on feeder cells are washed two times with PBS (-), subjected to trypsinization with a 0.05% trypsin liquid for 2 minutes, and scraped from the petri dish to yield a mass of several tens of the cells. ES medium (-G418) is added to the cells to inactivate trypsin, and after centrifugation, the cells are suspended in ES medium. They are seeded in a petri dish covered with 0.1% gelatin, and allowed to stand for 15 minutes in a CO<sub>2</sub> incubator. Cells not adhered to the dish are collected, and these cells are used for preparation of an aggregated chimera.

The adhesion between the 2.5 days embryos and ES cells is performed as follows. First, wells are made on the bottom of a petri dish for bacterial culture using a needle. The well is covered with a drop of 20  $\mu$ l of BWV medium, and further covered with mineral oil. Then, the dish is placed in a CO<sub>2</sub> incubator, thus the medium is equilibrated to the condition. The masses consisting of 10-20 ES cells having a good shape are selected under a stereoscopic microscope. Those masses of ES cells are placed into the wells prepared as above one by one. The 2.5 days embryos of which clear zones have been removed are placed into the wells at 2 embryos per well to achieve adhesion to the mass of ES cells. The dish is cultured overnight in a CO<sub>2</sub> incubator, and one blastocyst is resulted having an almost equivalent size to that of 2 pieces.

Next, 3.5 days embryos are transplanted into uterus. Nembutal parenteral solution is administered intraperitoneally (50  $\mu$ g per 1g of body weight) to a pseudopregnant mouse (2.5 days after mating) for anesthetization. The dorsal side of the anesthetized mouse is dissected, and the uterus is extracted through clutching fat mass. The blastocysts or morulas prepared by culturing overnight are sucked up along with a small amount of medium (0.5-1.0  $\mu$ l) at 10-15 pieces each to a pipet, thereafter, those embryos are transplanted into a uterine by puncturing the uterine wall at a part close to the uterine tube with a needle of syringe, and inserting a pipet through the opening followed by ejection. The uterine is returned within the body, and the opening is sutured, subsequently, approximately 18 days later, an infant is born.

After transmission to the germ line is confirmed, the mice which are heterologous each other are crossed to result in homogenization, accordingly, an objective knockout mouse that is not expressing a homologue of a novel collectin of



the present invention is obtained.

[Industrial Applicability]

As set forth above, a novel collectin and a gene encoding the novel collectin having characteristic structures of the collectins that have been demonstrated to exhibit anti-bacterial, anti-viral activities, which are different from the collectins reported so far, are provided by the present invention.

The sequence information presented by the present invention is useful for investigating mechanisms of biological defense systems, and may provide medical, experimental tools in which biological activities of the novel collectin are utilized. For example, vectors that can express the novel collectin, host cells comprising the vector with feasibility of expression, antibodies for the novel collectin, as well as probes for screening the related molecular species of the novel collectin can be provided.

In addition, transgenic non-human animals, and knockout non-human animals are provided, which may be utilized as disease model animals for studies on functions, or regulation of expression of the novel collectin and/or the derived molecules, for elucidating the mechanisms involved in diseases related to the cells where those molecules are expressed in human, and for screening and safety test of pharmaceutical products.

**WHAT IS CLAIMED IS:**

1. An isolated polynucleotide comprising the nucleotide sequence that encodes a protein having the following amino acid sequence:

5 Met-Gln-Gln-Asp-Leu-Met-Arg-Ser-Arg-Leu-Asp-Thr-Glu-Val-Ala-Asn-  
Leu-Ser-Val-Ile-Met-Glu-Glu-Met-Lys-Leu-Val-Asp-Ser-Lys-His-Gly-  
Gln-Leu-Ile-Lys-Asn-Phe-Thr-Ile-Leu-Gln-Gly-Pro-Pro-Gly-Pro-Arg-  
Gly-Pro-Arg-Gly-Asp-Arg-Gly-Ser-Gln-Gly-Pro-Pro-Gly-Pro-Thr-Gly-  
Asn-Lys-Gly-Gln-Lys-Gly-Glu-Lys-Gly-Glu-Pro-Gly-Pro-Pro-Gly-Pro-  
10 Ala-Gly-Glu-Arg-Gly-Pro-Ile-Gly-Pro-Ala-Gly-Pro-Pro-Gly-Glu-Arg-  
Gly-Gly-Lys-Gly-Ser-Lys-Gly-Ser-Gln-Gly-Pro-Lys-Gly-Ser-Arg-Gly-  
Ser-Pro-Gly-Lys-Pro-Gly-Pro-Gln-Gly-Pro-Ser-Gly-Asp-Pro-Gly-Pro-  
Pro-Gly-Pro-Pro-Gly-Lys-Glu-Gly-Leu-Pro-Gly-Pro-Gln-Gly-Pro-Pro-  
Gly-Phe-Gln-Gly-Leu-Gln-Gly-Thr-Val-Gly-Glu-Pro-Gly-Val-Pro-Gly-  
15 Pro-Arg-Gly-Leu-Pro-Gly-Leu-Pro-Gly-Val-Pro-Gly-Met-Pro-Gly-Pro-  
Lys-Gly-Pro-Pro-Gly-Pro-Pro-Gly-Pro-Ser-Gly-Ala-Val-Val-Pro-Leu-  
Ala-Leu-Gln-Asn-Glu-Pro-Thr-Pro-Ala-Pro-Glu-Asp-Asn-Gly-Cys-Pro-  
Pro-His-Trp-Lys-Asn-Phe-Thr-Asp-Lys-Cys-Tyr-Tyr-Phe-Ser-Val-Glu-  
Lys-Glu-Ile-Phe-Glu-Asp-Ala-Lys-Leu-Phe-Cys-Glu-Asp-Lys-Ser-Ser-  
20 His-Leu-Val-Phe-Ile-Asn-Thr-Arg-Glu-Glu-Gln-Gln-Trp-Ile-Lys-Lys-  
Gln-Met-Val-Gly-Arg-Glu-Ser-His-Trp-Ile-Gly-Leu-Thr-Asp-Ser-Glu-  
Arg-Glu-Asn-Glu-Trp-Lys-Trp-Leu-Asp-Gly-Thr-Ser-Pro-Asp-Tyr-Lys-  
Asn-Trp-Lys-Ala-Gly-Gln-Pro-Asp-Asn-Trp-Gly-His-Gly-His-Gly-Pro-  
Gly-Glu-Asp-Cys-Ala-Gly-Leu-Ile-Tyr-Ala-Gly-Gln-Trp-Asn-Asp-Phe-  
25 Gln-Cys-Glu-Asp-Val-Asn-Asn-Phe-Ile-Cys-Glu-Lys-Asp-Arg-Glu-Thr-  
Val-Leu-Ser-Ser-Ala-Leu (SEQ ID NO:2, 206-547).

2. An isolated polynucleotide comprising the nucleotide sequence that

encodes a protein having the following amino acid sequence:

Met-Lys-Leu-Val-Asp-Ser-Lys-His-Gly-Gln-Leu-Ile-Lys-Asn-Phe-Thr-  
Ile-Leu-Gln-Gly-Pro-Pro-Gly-Pro-Arg-Gly-Pro-Arg-Gly-Asp-Arg-Gly-  
Ser-Gln-Gly-Pro-Pro-Gly-Pro-Thr-Gly-Asn-Lys-Gly-Gln-Lys-Gly-Glu-  
5 Lys-Gly-Glu-Pro-Gly-Pro-Pro-Gly-Pro-Ala-Gly-Glu-Arg-Gly-Pro-Ile-  
Gly-Pro-Ala-Gly-Pro-Pro-Gly-Glu-Arg-Gly-Gly-Lys-Gly-Ser-Lys-Gly-  
Ser-Gln-Gly-Pro-Lys-Gly-Ser-Arg-Gly-Ser-Pro-Gly-Lys-Pro-Gly-Pro-  
Gln-Gly-Pro-Ser-Gly-Asp-Pro-Gly-Pro-Pro-Gly-Pro-Pro-Gly-Lys-Glu-  
Gly-Leu-Pro-Gly-Pro-Gln-Gly-Pro-Pro-Gly-Phe-Gln-Gly-Leu-Gln-Gly-  
10 Thr-Val-Gly-Glu-Pro-Gly-Val-Pro-Gly-Pro-Arg-Gly-Leu-Pro-Gly-Leu-  
Pro-Gly-Val-Pro-Gly-Met-Pro-Gly-Pro-Lys-Gly-Pro-Pro-Gly-Pro-Pro-  
Gly-Pro-Ser-Gly-Ala-Val-Val-Pro-Leu-Ala-Leu-Gln-Asn-Glu-Pro-Thr-  
Pro-Ala-Pro-Glu-Asp-Asn-Gly-Cys-Pro-Pro-His-Trp-Lys-Asn-Phe-Thr-  
Asp-Lys-Cys-Tyr-Tyr-Phe-Ser-Val-Glu-Lys-Glu-Ile-Phe-Glu-Asp-Ala-  
15 Lys-Leu-Phe-Cys-Glu-Asp-Lys-Ser-Ser-His-Leu-Val-Phe-Ile-Asn-Thr-  
Arg-Glu-Glu-Gln-Gln-Trp-Ile-Lys-Lys-Gln-Met-Val-Gly-Arg-Glu-Ser-  
His-Trp-Ile-Gly-Leu-Thr-Asp-Ser-Glu-Arg-Glu-Asn-Glu-Trp-Lys-Trp-  
Leu-Asp-Gly-Thr-Ser-Pro-Asp-Tyr-Lys-Asn-Trp-Lys-Ala-Gly-Gln-Pro-  
Asp-Asn-Trp-Gly-His-Gly-His-Gly-Pro-Gly-Glu-Asp-Cys-Ala-Gly-Leu-  
20 Ile-Tyr-Ala-Gly-Gln-Trp-Asn-Asp-Phe-Gln-Cys-Glu-Asp-Val-Asn-Asn-  
Phe-Ile-Cys-Glu-Lys-Asp-Arg-Glu-Thr-Val-Leu-Ser-Ser-Ala-Leu (SEQ ID N  
O: 2, 229-547).

3. The polynucleotide according to claim 2, wherein said protein fur-  
25 ther comprises the following amino acid sequence upstream of the first methioni-  
ne residue (SEQ ID NO:2, 229):

Met-Glu-Glu (SEQ ID NO: 2, 226-228); or

Met-Arg-Ser-Arg-Leu-Asp-Thr-Glu-Val-Ala-Asn-Leu-Ser-Val-Ile-Met-Glu-Glu (SEQ ID NO: 2, 211-228).

4. The polynucleotide according to claim 2, wherein said protein further comprises the following amino acid sequence upstream of the first methionine residue (SEQ ID NO:2, 229):

Met-Glu-Asn-Ile-Thr-Thr-Ile-Ser-Gln-Ala-Asn-Glu-Gln-Asn-Leu-Lys-Asp-Leu-Gln-Asp-Leu-His-Lys-Asp-Ala-Glu-Asn-Arg-Thr-Ala-Ile-Lys-Phe-Asn-Gln-Leu-Glu-Glu-Arg-Phe-Gln-Leu-Phe-Glu-Thr-Asp-Ile-Val-Asn-Ile-Ile-Ser-Asn-Ile-Ser-Tyr-Thr-Ala-His-His-Leu-Arg-Thr-Leu-Thr-Ser-Asn-Leu-Asn-Glu-Val-Arg-Thr-Thr-Cys-Thr-Asp-Thr-Leu-Thr-Lys-His-Thr-Asp-Asp-Leu-Thr-Ser-Leu-Asn-Asn-Thr-Leu-Ala-Asn-Ile-Arg-Leu-Asp-Ser-Val-Ser-Leu-Arg-Met-Gln-Gln-Asp-Leu-Met-Arg-Ser-Arg-Leu-Asp-Thr-Glu-Val-Ala-Asn-Leu-Ser-Val-Ile-Met-Glu-Glu (SEQ ID NO: 102-228);

Met-Asn-Ser-Gln-Leu-Asn-Ser-Phe-Thr-Gly-Gln-Met-Glu-Asn-Ile-Thr-Thr-Ile-Ser-Gln-Ala-Asn-Glu-Gln-Asn-Leu-Lys-Asp-Leu-Gln-Asp-Leu-His-Lys-Asp-Ala-Glu-Asn-Arg-Thr-Ala-Ile-Lys-Phe-Asn-Gln-Leu-Glu-Glu-Arg-Phe-Gln-Leu-Phe-Glu-Thr-Asp-Ile-Val-Asn-Ile-Ile-Ser-Asn-Ile-Ser-Tyr-Thr-Ala-His-His-Leu-Arg-Thr-Leu-Thr-Ser-Asn-Leu-Asn-Glu-Val-Arg-Thr-Thr-Cys-Thr-Asp-Thr-Leu-Thr-Lys-His-Thr-Asp-Asp-Leu-Thr-Ser-Leu-Asn-Asn-Thr-Leu-Ala-Asn-Ile-Arg-Leu-Asp-Ser-Val-Ser-Leu-Arg-Met-Gln-Gln-Asp-Leu-Met-Arg-Ser-Arg-Leu-Asp-Thr-Glu-Val-Ala-Asn-Leu-Ser-Val-Ile-Met-Glu-Glu (SEQ ID NO: 2, 91-228);

Met-Asn-Leu-Asn-Asn-Leu-Asn-Leu-Thr-Gln-Val-Gln-Gln-Arg-Asn-Leu-

Ile-Thr-Asn-Leu-Gln-Arg-Ser-Val-Asp-Asp-Thr-Ser-Gln-Ala-Ile-Gln-  
Arg-Ile-Lys-Asn-Asp-Phe-Gln-Asn-Leu-Gln-Gln-Val-Phe-Leu-Gln-Ala-  
Lys-Lys-Asp-Thr-Asp-Trp-Leu-Lys-Glu-Lys-Val-Gln-Ser-Leu-Gln-Thr-  
Leu-Ala-Ala-Asn-Asn-Ser-Ala-Leu-Ala-Lys-Ala-Asn-Asn-Asp-Thr-Leu-  
5 Glu-Asp-Met-Asn-Ser-Gln-Leu-Asn-Ser-Phe-Thr-Gly-Gln-Met-Glu-Asn-  
Ile-Thr-Thr-Ile-Ser-Gln-Ala-Asn-Glu-Gln-Asn-Leu-Lys-Asp-Leu-Gln-  
Asp-Leu-His-Lys-Asp-Ala-Glu-Asn-Arg-Thr-Ala-Ile-Lys-Phe-Asn-Gln-  
Leu-Glu-Glu-Arg-Phe-Gln-Leu-Phe-Glu-Thr-Asp-Ile-Val-Asn-Ile-Ile-  
Ser-Asn-Ile-Ser-Tyr-Thr-Ala-His-His-Leu-Arg-Thr-Leu-Thr-Ser-Asn-  
10 Leu-Asn-Glu-Val-Arg-Thr-Thr-Cys-Thr-Asp-Thr-Leu-Thr-Lys-His-Thr-  
Asp-Asp-Leu-Thr-Ser-Leu-Asn-Asn-Thr-Leu-Ala-Asn-Ile-Arg-Leu-Asp-  
Ser-Val-Ser-Leu-Arg-Met-Gln-Gln-Asp-Leu-Met-Arg-Ser-Arg-Leu-Asp-  
Thr-Glu-Val-Ala-Asn-Leu-Ser-Val-Ile-Met-Glu-Glu (SEQ ID NO: 2, 9-228);

or

15 Met-Tyr-Ser-His-Asn-Val-Val-Ile-Met-Asn-Leu-Asn-Asn-Leu-Asn-Leu-  
Thr-Gln-Val-Gln-Gln-Arg-Asn-Leu-Ile-Thr-Asn-Leu-Gln-Arg-Ser-Val-  
Asp-Asp-Thr-Ser-Gln-Ala-Ile-Gln-Arg-Ile-Lys-Asn-Asp-Phe-Gln-Asn-  
Leu-Gln-Gln-Val-Phe-Leu-Gln-Ala-Lys-Lys-Asp-Thr-Asp-Trp-Leu-Lys-  
Glu-Lys-Val-Gln-Ser-Leu-Gln-Thr-Leu-Ala-Ala-Asn-Asn-Ser-Ala-Leu-  
20 Ala-Lys-Ala-Asn-Asn-Asp-Thr-Leu-Glu-Asp-Met-Asn-Ser-Gln-Leu-Asn-  
Ser-Phe-Thr-Gly-Gln-Met-Glu-Asn-Ile-Thr-Thr-Ile-Ser-Gln-Ala-Asn-  
Glu-Gln-Asn-Leu-Lys-Asp-Leu-Gln-Asp-Leu-His-Lys-Asp-Ala-Glu-Asn-  
Arg-Thr-Ala-Ile-Lys-Phe-Asn-Gln-Leu-Glu-Glu-Arg-Phe-Gln-Leu-Phe-  
Glu-Thr-Asp-Ile-Val-Asn-Ile-Ile-Ser-Asn-Ile-Ser-Tyr-Thr-Ala-His-  
25 His-Leu-Arg-Thr-Leu-Thr-Ser-Asn-Leu-Asn-Glu-Val-Arg-Thr-Thr-Cys-  
Thr-Asp-Thr-Leu-Thr-Lys-His-Thr-Asp-Asp-Leu-Thr-Ser-Leu-Asn-Asn-

Thr-Leu-Ala-Asn-Ile-Arg-Leu-Asp-Ser-Val-Ser-Leu-Arg-Met-Gln-Gln-  
Asp-Leu-Met-Arg-Ser-Arg-Leu-Asp-Thr-Glu-Val-Ala-Asn-Leu-Ser-Val-  
Ile-Met-Glu-Glu (SEQ ID NO: 2, 1-228).

- 5                    5.    An isolated polynucleotide comprising a nucleotide sequence having  
the following nucleotide sequence:

atgcaacaag atttgatgag gtcgaggtta gacactgaag tagccaactt atcagtgatt  
atggaagaaa tgaagctagt agactccaag catggtcagc tcatacaagaa tttacaata  
ctacaaggtc caccggggccc caggggtcca agaggtgaca gaggatccca gggacccctt  
ggcccaactg gcaacaaggg acagaaagga gagaaggggg agcctggacc acctggccct  
gcgggtgaga gaggcccaat tggaccagct ggtcccccg gagagcgtgg cggcaaagga  
tctaaaggct ccaggggccc caaaggctcc cgtggttccc ctgggaagcc cggccctcag  
ggccccagt gggaccagg cccccgggc ccaccaggca aagagggact cccccgccct  
cagggccctc ctggttcca gggacttcag ggcaccgttg gggagcctgg ggtgcctgga  
cctcggggac tgccaggctt gcctggggta ccaggcatgc caggcccca gggccccccc  
ggccctctg gcccatcagg agcgggtggtg cccctggccc tgcagaatga gccaaccccg  
gcaccggagg acaatggctg cccgcctcac tggagaact tcacagacaa atgctactat  
ttttcagttg agaaagaaat tttgaggat gcaaagcttt tctgtgaaga caagtcttca  
catctgttt tcataaacac tagagaggaa cagcaatgga taaaaaaca gatggtaggg  
20 agagagagcc actggatcgg cctcacagac tcagagcgtg aaaatgaatg gaagtggctg  
gatgggacat ctccagacta caaaaattgg aaagctggac agccggataa ctggggtcac  
ggccatgggc caggagaaga ctgtgctggg ttgatttatg ctgggcagtg gaacgatttc  
caatgtgaag acgtcaataa ctctatttgc gaaaaagaca gggagacagt actgtcatct  
gcatta (SEQ ID NO: 1, 670-1695).

25

6.    An isolated polynucleotide comprising a nucleotide sequence having

the following nucleotide sequence:

atgaagctag tagactccaa gcatggtcag ctcataaga attttacaat actacaaggt  
ccaccggggc ccaggggtcc aagaggtgac agaggatccc agggaccccc tggcccaact  
ggcaacaagg gacagaaagg agagaagggg gagcctggac cacctggccc tgcgggtgag  
5 agaggcccaa ttggaccagc tggtcceccc ggagagcgtg gcggcaagg atctaaaggc  
tcccagggcc ccaaaggctc cgtgtgttcc cctgggaagc cggccctca gggccccagt  
ggggaccacc gcccccggg ccaccaggc aaagaggac tcccggccc tcaggccct  
cctggttcc agggacttca gggaccgtt ggggagcctg ggtgctgg acctcgggga  
ctgccaggct tgcctgggt accaggcatg ccaggcccca agggccccc cggccctct  
10 ggcccatcag gagcgtgtt gccctggcc ctgcagaatg agccaacccc ggcaccggag  
gacaatggct gcccgctca ctggaagaac ttacagaca aatgtacta ttttcagtt  
gagaaagaaa tttttgagga tgcaaagctt ttctgtgaag acaagtcttc acatctgtt  
ttcataaaca ctagagagga acagcaatgg ataaaaaac agatggtagg gagagagac  
cactggatcg gctcacaga ctcagagcgt gaaaatgaat ggaagtggct gcatgggaca  
15 totccagact acaaaaattg gaaagctgga cagccggata actgggtca tggccatggg  
ccaggagaag actgtgctgg gttgatttat gctgggcagt ggaacgattt ccaatgtgaa  
gacgtcaata acttcatttg cgaaaaagac agggagacag tactgtcatc tgcatta  
(SEQ ID NO: 1, 739-1695).

7. The polynucleotide according to claim 6 further comprises the following nucleotide sequence 5' upstream of said nucleotide sequence:

atggaagaa (SEQ ID NO: 1, 730-738); or

atgaggtcga ggttagacac tgaagtagcc aacttatcag tgattatgga agaa (SEQ ID NO: 1, 685-738).

8. The polynucleotide according to claim 6 further comprises the following

nucleotide sequence 5' upstream of said nucleotide sequence:

atggagaaca tcaccactat ctctcaagcc aacgagcaga acctgaaaga cctgcaggac  
ttacacaaag atgcagagaa tagaacagcc atcaagtcca accaactgga ggaacgcttc  
cagctctttg agacggatat tgtgaacatc attagcaata tcagttacac agcccaccac  
5 ctgcggagcg tgaccagcaa tctaaatgaa gtcaggacca cttgcacaga tacctttacc  
aaacacacag atgatctgac ctcttgaat aataccctgg ccaacatccg ttggattct  
gtttctctca ggatgaaca agatttgatg aggtcgaggt tagacactga agtagccaac  
ttatcagtga ttatggaaga a (SEQ ID NO: 1, 358-738);

atgaacagcc agctcaactc attcacaggt cagatggaga acatcaccac tatctctcaa  
100 gccaacgagc agaacctgaa agacctgcag gacttacaca aagatgcaga gaatagaaca  
gccatcaagt tcaaccaact ggaggaacgc ttccagctct ttgagacgga tatttgtaac  
atcattagca atatcagtta cacagcccac cacctgcgga cgctgaccag caatctaaat  
gaagtcagga ccaattgcac agataccctt accaaacaca cagatgatct gacctctctg  
aataataccc tggccaacat cgttttgat tctgtttctc tcaggatgca acaagatttg  
15 atgaggtcga ggtagacac tgaagtagcc aacttatcag tgattatgga agaa (SEQ ID NO:  
1, 325-738);

atgaacctca acaacctgaa cctgaccagc gtgcagcaga ggaacctcat cacgaatctg  
cagcggctctg tggatgacac aagccaggct atccagcgaa tcaagaacga ctttcaaagt  
ctgcagcagg tttttcttca agccaagaag gacacggatt ggctgaagga gaaagtgcag  
20 agcttgacga cgctggctgc caacaactct gcgttgGCCa aagccaacaa cgacacctg  
gaggatatga acagccagct caactcattc acaggtcaga tggagaacat caccactatc  
tctcaagcca acgagcagaa cctgaaagac ctgcaggact tacacaaaga tgcagagaat  
agaacagcca tcaagttcaa ccaactggag gaacgcttcc agctctttga gacggatat  
gtgaacatca ttagcaatat cagttacaca gccaccacc tgcggagcgt gaccagcaat  
25 ctaaatgaag tcaggaccac ttgcacagat acccttacca aacacacaga tgatctgacc  
tccttgaata ataccctggc caacatccgt ttggattctg tttctctcag gatgcaacaa



gatttgatga ggctcgaggtt agacactgaa gtagccaact tatcagtgat tatggaagaa (SEQ I  
D NO: 1, 79-738);

atgtattctc ataattgtgt catcatgaac ctcaacaacc tgaacctgac ccaggtgcag  
cagaggaacc tcatcacgaa tctgcagcgg tctgtgatg acacaagcca ggctatccag  
5 cgaatcaaga acgactttca aaatctgcag caggtttttc ttcaagccaa gaaggacagc  
gattggctga aggagaaagt gcagagcttg cagacgttg ctgccaacaa ctctgcgttg  
gccaaagcca acaacgacac cctggaggat atgaacagcc agctcaactc attcacaggt  
cagatggaga acatcaccac tatctctcaa gccaacgagc agaacctgaa agacctgcag  
gacttacaca aagatgcaga gaatagaaca gccatcaagt tcaaccaact ggaggaacgc  
10 ttccagctct ttgagacgga tattgtgaac atcattagca atatcagtta cacagccac  
cacctgcgga cgctgaccag caatctaaat gaagtcagga ccacttgac agataccctt  
accaaacaca cagatgatct gacctccttg aataataccc tggccaacat cgtttggat  
tctgtttctc tcaggatgca acaagatttg atgaggtcga ggtagacac tgaagtagcc  
aacttatcag tgattatgga agaa (SEQ ID NO:1, 55-738); or

gtcacgaatc tgcagaaga taccagcgtg ctccaggcca atctgcagaa ccaaattgat  
tctcataatg tggctcatcat gaacctcaac aacctgaacc tgacccaggt gcagcagagg  
15 aacctcatca cgaatctgca gcggtctgtg gatgacacaa gccaggctat ccagcgaatc  
aagaacgact ttcaaaatct gcagcaggtt tttcttcaag ccaagaagga caccgattgg  
ctgaaggaga aagtgcagag cttgcagacg ctggctgcc acaactctgc gttggccaaa  
20 gccacaacg acaccttgga ggatatgaac agccagctca actcattcac aggtcagatg  
gagaacatca ccactatctc tcaagccaac gacgagaacc tgaagacct gcaggactta  
cacaagatg cagagaatag aacagccatc aagttaacc aactggagga acgcttccag  
ctctttgaga cggatattgt gaacatcatt agcaatatca gttacacagc ccaccacctg  
cggacgtga ccagcaatct aaatgaagtc aggaccactt gcacagatac cttaccaaaa  
25 cacacagatg atctgacctc cttgaataat accctggcca acatccgttt ggattctgtt  
tctctcagga tgcaacaaga tttgatgagg tcgaggttag aactgaagt agccaactta

tcagtgatta tggaagaa (SEQ ID NO: 1, 1-738).

9. The polynucleotide according to any one of claims 5 to 8 further comprises the following nucleotide sequence 3' downstream of said nucleotide sequence:

taacggactg tgatgggac acatgagcaa atttcagct ctcaaagca aaggacactc  
ctttctaatt gcatcacctt ctcacagat tgaaaaaaaa aaagcactg aaaaccaatt  
actgaaaaaa aattgacagc tagtgTTTT taccatcctg cattacccaa agacttgga  
actaaaatgt tccccagggt gatatgctga ttttcattgt gcacatggac tgaatcacat  
agattctcct ccgtcagtaa ccgtgcgatt atacaaatta tgtcttccaa agtatggaac  
actccaatga gaaaaggtt atcatccg (SEQ ID NO: 1, 1696-2024).

10. An isolated polynucleotide encoding a novel collectin, which can hybridize under a stringent condition with a probe that is an amplification product from PCR reaction performed using primers having the following nucleotide sequences:

caatctgatgagaaggtgatg (SEQ ID NO: 4) and  
acgaggggctggatgggacat (SEQ ID NO: 5).

11. A polynucleotide which can hybridize under a stringent condition with any one of the polynucleotide according to any one of claims 1 to 10, wherein the protein encoded by said polynucleotide is a novel collectin which comprises: (1)  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and (2) collagen-like region.

12. The polynucleotide according to any one of claims 1 to 11, wherein said polynucleotide is cDNA.

13. A novel collectin comprising the amino acid sequence encoded by the polynucleotide according to any one of claims 5 to 12.

14. A novel collectin comprising the amino acid sequence:

Met-Gln-Gln-Asp-Leu-Met-Arg-Ser-Arg-Leu-Asp-Thr-Glu-Val-Ala-Asn-  
Leu-Ser-Val-Ile-Met-Glu-Glu-Met-Lys-Leu-Val-Asp-Ser-Lys-His-Gly-  
Gln-Leu-Ile-Lys-Asn-Phe-Thr-Ile-Leu-Gln-Gly-Pro-Pro-Gly-Pro-Arg-  
Gly-Pro-Arg-Gly-Asp-Arg-Gly-Ser-Gln-Gly-Pro-Pro-Gly-Pro-Thr-Gly-  
5 Asn-Lys-Gly-Gln-Lys-Gly-Glu-Lys-Gly-Glu-Pro-Gly-Pro-Pro-Gly-Pro-  
Ala-Gly-Glu-Arg-Gly-Pro-Ile-Gly-Pro-Ala-Gly-Pro-Pro-Gly-Glu-Arg-  
Gly-Gly-Lys-Gly-Ser-Lys-Gly-Ser-Gln-Gly-Pro-Lys-Gly-Ser-Arg-Gly-  
Ser-Pro-Gly-Lys-Pro-Gly-Pro-Gln-Gly-Pro-Ser-Gly-Asp-Pro-Gly-Pro-  
Pro-Gly-Pro-Pro-Gly-Lys-Glu-Gly-Leu-Pro-Gly-Pro-Gln-Gly-Pro-Pro-  
10 Gly-Phe-Gln-Gly-Leu-Gln-Gly-Thr-Val-Gly-Glu-Pro-Gly-Val-Pro-Gly-  
Pro-Arg-Gly-Leu-Pro-Gly-Leu-Pro-Gly-Val-Pro-Gly-Met-Pro-Gly-Pro-  
Lys-Gly-Pro-Pro-Gly-Pro-Pro-Gly-Pro-Ser-Gly-Ala-Val-Val-Pro-Leu-  
Ala-Leu-Gln-Asn-Glu-Pro-Thr-Pro-Ala-Pro-Glu-Asp-Asn-Gly-Cys-Pro-  
Pro-His-Trp-Lys-Asn-Phe-Thr-Asp-Lys-Cys-Tyr-Tyr-Phe-Ser-Val-Glu-  
15 Lys-Glu-Ile-Phe-Glu-Asp-Ala-Lys-Leu-Phe-Cys-Glu-Asp-Lys-Ser-Ser-  
His-Leu-Val-Phe-Ile-Asn-Thr-Arg-Glu-Glu-Gln-Gln-Trp-Ile-Lys-Lys-  
Gln-Met-Val-Gly-Arg-Glu-Ser-His-Trp-Ile-Gly-Leu-Thr-Asp-Ser-Glu-  
Arg-Glu-Asn-Glu-Trp-Lys-Trp-Leu-Asp-Gly-Thr-Ser-Pro-Asp-Tyr-Lys-  
Asn-Trp-Lys-Ala-Gly-Gln-Pro-Asp-Asn-Trp-Gly-His-Gly-His-Gly-Pro-  
20 Gly-Glu-Asp-Cys-Ala-Gly-Leu-Ile-Tyr-Ala-Gly-Gln-Trp-Asn-Asp-Phe-  
Gln-Cys-Glu-Asp-Val-Asn-Asn-Phe-Ile-Cys-Glu-Lys-Asp-Arg-Glu-Thr-  
Val-Leu-Ser-Ser-Ala-Leu (SEQ ID NO: 2, 206-547).

15. A novel collectin comprising the amino acid sequence:

25 Met-Lys-Leu-Val-Asp-Ser-Lys-His-Gly-Gln-Leu-Ile-Lys-Asn-Phe-Thr-  
Ile-Leu-Gln-Gly-Pro-Pro-Gly-Pro-Arg-Gly-Pro-Arg-Gly-Asp-Arg-Gly-

Ser-Gln-Gly-Pro-Pro-Gly-Pro-Thr-Gly-Asn-Lys-Gly-Gln-Lys-Gly-Glu-  
 Lys-Gly-Glu-Pro-Gly-Pro-Pro-Gly-Pro-Ala-Gly-Glu-Arg-Gly-Pro-Ile-  
 Gly-Pro-Ala-Gly-Pro-Pro-Gly-Glu-Arg-Gly-Gly-Lys-Gly-Ser-Lys-Gly-  
 Ser-Gln-Gly-Pro-Lys-Gly-Ser-Arg-Gly-Ser-Pro-Gly-Lys-Pro-Gly-Pro-  
 5 Gln-Gly-Pro-Ser-Gly-Asp-Pro-Gly-Pro-Pro-Gly-Pro-Pro-Gly-Lys-Glu-  
 Gly-Leu-Pro-Gly-Pro-Gln-Gly-Pro-Pro-Gly-Phe-Gln-Gly-Leu-Gln-Gly-  
 Thr-Val-Gly-Glu-Pro-Gly-Val-Pro-Gly-Pro-Arg-Gly-Leu-Pro-Gly-Leu-  
 Pro-Gly-Val-Pro-Gly-Met-Pro-Gly-Pro-Lys-Gly-Pro-Pro-Gly-Pro-Pro-  
 Gly-Pro-Ser-Gly-Ala-Val-Val-Pro-Leu-Ala-Leu-Gln-Asn-Glu-Pro-Thr-  
 10 Pro-Ala-Pro-Glu-Asp-Asn-Gly-Cys-Pro-Pro-His-Trp-Lys-Asn-Phe-Thr-  
 Asp-Lys-Cys-Tyr-Tyr-Phe-Ser-Val-Glu-Lys-Glu-Ile-Phe-Glu-Asp-Ala-  
 Lys-Leu-Phe-Cys-Glu-Asp-Lys-Ser-Ser-His-Leu-Val-Phe-Ile-Asn-Thr-  
 Arg-Glu-Glu-Gln-Gln-Trp-Ile-Lys-Lys-Gln-Met-Val-Gly-Arg-Glu-Ser-  
 His-Trp-Ile-Gly-Leu-Thr-Asp-Ser-Glu-Arg-Glu-Asn-Glu-Trp-Lys-Trp-  
 15 Leu-Asp-Gly-Thr-Ser-Pro-Asp-Tyr-Lys-Asn-Trp-Lys-Ala-Gly-Gln-Pro-  
 Asp-Asn-Trp-Gly-His-Gly-His-Gly-Pro-Gly-Glu-Asp-Cys-Ala-Gly-Leu-  
 Ile-Tyr-Ala-Gly-Gln-Trp-Asn-Asp-Phe-Gln-Cys-Glu-Asp-Val-Asn-Asn-  
 Phe-Ile-Cys-Glu-Lys-Asp-Arg-Glu-Thr-Val-Leu-Ser-Ser-Ala-Leu (SEQ ID NO: 2,  
 229-547).

20 16. The novel collectin according to claim 15, wherein said novel collectin  
 further comprises the following amino acid sequence upstream of the first methionine  
 residue (SEQ ID NO: 2, 229):

Met-Glu-Glu (SEQ ID NO: 2, 226-228); or

25 Met-Arg-Ser-Arg-Leu-Asp-Thr-Glu-Val-Ala-Asn-Leu-Ser-Val-Ile-Met-Glu-Glu (SE  
 Q ID NO: 2, 211-228).

17. The novel collectin according to claim 15, wherein said novel collectin further comprises the following amino acid sequence upstream of the first methionine residue (SEQ ID NO: 2, 229):

Met-Glu-Asn-Ile-Thr-Thr-Ile-Ser-Gln-Ala-Asn-Glu-Gln-Asn-Leu-Lys-  
Asp-Leu-Gln-Asp-Leu-His-Lys-Asp-Ala-Glu-Asn-Arg-Thr-Ala-Ile-Lys-  
Phe-Asn-Gln-Leu-Glu-Glu-Arg-Phe-Gln-Leu-Phe-Glu-Thr-Asp-Ile-Val-  
Asn-Ile-Ile-Ser-Asn-Ile-Ser-Tyr-Thr-Ala-His-His-Leu-Arg-Thr-Leu-  
Thr-Ser-Asn-Leu-Asn-Glu-Val-Arg-Thr-Thr-Cys-Thr-Asp-Thr-Leu-Thr-  
Lys-His-Thr-Asp-Asp-Leu-Thr-Ser-Leu-Asn-Asn-Thr-Leu-Ala-Asn-Ile-  
Arg-Leu-Asp-Ser-Val-Ser-Leu-Arg-Met-Gln-Gln-Asp-Leu-Met-Arg-Ser-  
Arg-Leu-Asp-Thr-Glu-Val-Ala-Asn-Leu-Ser-Val-Ile-Met-Glu-Glu (SEQ ID NO: 2,  
102-228);

Met-Asn-Ser-Gln-Leu-Asn-Ser-Phe-Thr-Gly-Gln-Met-Glu-Asn-Ile-Thr-  
Thr-Ile-Ser-Gln-Ala-Asn-Glu-Gln-Asn-Leu-Lys-Asp-Leu-Gln-Asp-Leu-  
His-Lys-Asp-Ala-Glu-Asn-Arg-Thr-Ala-Ile-Lys-Phe-Asn-Gln-Leu-Glu-  
Glu-Arg-Phe-Gln-Leu-Phe-Glu-Thr-Asp-Ile-Val-Asn-Ile-Ile-Ser-Asn-  
Ile-Ser-Tyr-Thr-Ala-His-His-Leu-Arg-Thr-Leu-Thr-Ser-Asn-Leu-Asn-  
Glu-Val-Arg-Thr-Thr-Cys-Thr-Asp-Thr-Leu-Thr-Lys-His-Thr-Asp-Asp-  
Leu-Thr-Ser-Leu-Asn-Asn-Thr-Leu-Ala-Asn-Ile-Arg-Leu-Asp-Ser-Val-  
Ser-Leu-Arg-Met-Gln-Gln-Asp-Leu-Met-Arg-Ser-Arg-Leu-Asp-Thr-Glu-  
Val-Ala-Asn-Leu-Ser-Val-Ile-Met-Glu-Glu (SEQ ID NO: 2, 91-228);  
Met-Asn-Leu-Asn-Asn-Leu-Asn-Leu-Thr-Gln-Val-Gln-Gln-Arg-Asn-Leu-  
Ile-Thr-Asn-Leu-Gln-Arg-Ser-Val-Asp-Asp-Thr-Ser-Gln-Ala-Ile-Gln-  
Arg-Ile-Lys-Asn-Asp-Phe-Gln-Asn-Leu-Gln-Gln-Val-Phe-Leu-Gln-Ala-  
Lys-Lys-Asp-Thr-Asp-Trp-Leu-Lys-Glu-Lys-Val-Gln-Ser-Leu-Gln-Thr-  
Leu-Ala-Ala-Asn-Asn-Ser-Ala-Leu-Ala-Lys-Ala-Asn-Asn-Asp-Thr-Leu-  
Glu-Asp-Met-Asn-Ser-Gln-Leu-Asn-Ser-Phe-Thr-Gly-Gln-Met-Glu-Asn-

Ile-Thr-Thr-Ile-Ser-Gln-Ala-Asn-Glu-Gln-Asn-Leu-Lys-Asp-Leu-Gln-  
 Asp-Leu-His-Lys-Asp-Ala-Glu-Asn-Arg-Thr-Ala-Ile-Lys-Phe-Asn-Gln-  
 Leu-Glu-Glu-Arg-Phe-Gln-Leu-Phe-Glu-Thr-Asp-Ile-Val-Asn-Ile-Ile-  
 Ser-Asn-Ile-Ser-Tyr-Thr-Ala-His-His-Leu-Arg-Thr-Leu-Thr-Ser-Asn-  
 5 Leu-Asn-Glu-Val-Arg-Thr-Thr-Cys-Thr-Asp-Thr-Leu-Thr-Lys-His-Thr-  
 Asp-Asp-Leu-Thr-Ser-Leu-Asn-Asn-Thr-Leu-Ala-Asn-Ile-Arg-Leu-Asp-  
 Ser-Val-Ser-Leu-Arg-Met-Gln-Gln-Asp-Leu-Met-Arg-Ser-Arg-Leu-Asp-  
 Thr-Glu-Val-Ala-Asn-Leu-Ser-Val-Ile-Met-Glu-Glu (SEQ ID NO: 2, 9-228); or  
 Met-Tyr-Ser-His-Asn-Val-Val-Ile-Met-Asn-Leu-Asn-Asn-Leu-Asn-Leu-  
 10 Thr-Gln-Val-Gln-Gln-Arg-Asn-Leu-Ile-Thr-Asn-Leu-Gln-Arg-Ser-Val-  
 Asp-Asp-Thr-Ser-Gln-Ala-Ile-Gln-Arg-Ile-Lys-Asn-Asp-Phe-Gln-Asn-  
 Leu-Gln-Gln-Val-Phe-Leu-Gln-Ala-Lys-Lys-Asp-Thr-Asp-Trp-Leu-Lys-  
 Glu-Lys-Val-Gln-Ser-Leu-Gln-Thr-Leu-Ala-Ala-Asn-Asn-Ser-Ala-Leu-  
 15 Ala-Lys-Ala-Asn-Asn-Asp-Thr-Leu-Glu-Asp-Met-Asn-Ser-Gln-Leu-Asn-  
 Ser-Phe-Thr-Gly-Gln-Met-Glu-Asn-Ile-Thr-Thr-Ile-Ser-Gln-Ala-Asn-  
 Glu-Gln-Asn-Leu-Lys-Asp-Leu-Gln-Asp-Leu-His-Lys-Asp-Ala-Glu-Asn-  
 Arg-Thr-Ala-Ile-Lys-Phe-Asn-Gln-Leu-Glu-Glu-Arg-Phe-Gln-Leu-Phe-  
 Glu-Thr-Asp-Ile-Val-Asn-Ile-Ile-Ser-Asn-Ile-Ser-Tyr-Thr-Ala-His-  
 His-Leu-Arg-Thr-Leu-Thr-Ser-Asn-Leu-Asn-Glu-Val-Arg-Thr-Thr-Cys-  
 20 Thr-Asp-Thr-Leu-Thr-Lys-His-Thr-Asp-Asp-Leu-Thr-Ser-Leu-Asn-Asn-  
 Thr-Leu-Ala-Asn-Ile-Arg-Leu-Asp-Ser-Val-Ser-Leu-Arg-Met-Gln-Gln-  
 Asp-Leu-Met-Arg-Ser-Arg-Leu-Asp-Thr-Glu-Val-Ala-Asn-Leu-Ser-Val-  
 Ile-Met-Glu-Glu (SEQ ID NO: 2, 1-228).

25 18. The novel collectin according to any one of claims 13 to 17, which is  
 derived from human.

19. The novel collectin consisting of the amino acid sequence that comprises deletion, substitution and/or addition of one or more amino acids in the collectin according to any one of claims 13 to 18, and said novel collectin comprises (1)  $\text{Ca}^{2+}$ -dependent carbohydrate recognition domain (CRD), and (2) collagen-like region.

20. A vector which comprises the polynucleotide according to any one of the claims 1 to 12 in a manner that allows expression of the novel collectin.

21. A host cell comprising the vector according to claim 20 in a manner that allows the expression.

22. A probe for screening the novel collectin related molecular species comprising the polynucleotide according to any one of claims 1 to 12 or a fragment thereof.

23. An antibody having specific immunoreactivity with the novel collectin according to any one of claims 13 to 19.

24. The antibody according to claim 23, which is a monoclonal antibody.

25. The antibody according to claim 23 or 24 having reduced immunogenicity to human.

26. A method for obtaining a novel collectin related molecular species, comprising screening a protein using the antibody according to any one of claims 23 to 25.

27. A method for obtaining a novel collectin and/or derived molecules, comprising screening a protein using the antibody according to any one of claims 23 to 25.

28. The method according to claim 26 or 27, wherein said screening is expression screening of cDNA library.

29. A method of quantitative determination of a novel collectin and/or derived molecules, comprising measuring an amount of the novel collectin and/or derived molecules contained in a test sample using the antibody according to any one of claims 23 to 25.

30. The method according to claim 29, wherein said antibody is employed in ELISA method.

31. A kit for quantitative determination of a novel collectin and/or derived molecules, comprising the antibody according to any one of claims 23 to 25, wherein an amount of the novel collectin and/or derived molecules in a test sample is measured by ELISA method using the antibody.

32. A method for obtaining a novel collectin and/or derived molecules, comprising isolating a novel collectin and/or derived molecules using the antibody according to any one of claims 23 to 25.

33. The method according to claim 32, wherein affinity chromatography by a support bound with said antibody, and/or immunoprecipitation is utilized.

34. The method according to claim 32 or 33, comprising expressing the novel collectin from the polynucleotide according to any one of claims 1 to 12 prior to the isolation step.

35. A transgenic non-human animal that is stably introduced with a recombinant gene comprising the polynucleotide according to any one of claims 1 to 12 into the chromosome, and that can express a novel collectin.

36. A transgenic non-human animal wherein introduction of a gene is effected in a non-human animal comprising a homologue of the novel collectin according to any one of claims 13 to 19, in a manner to modify the gene expression of said homologue.



37. A knockout non-human animal that is obtained by altering a gene encoding a homologue of the novel collectin according to any one of claims 13 to 19 in a non-human animal comprising said homologue, in a manner to inhibit the expression of said homologue.

## ABSTRACT

Novel collectin related molecules i.e., a novel collectin gene comprising a nucleotide sequence set out in SEQ ID NO: 1, and a novel collectin comprising an amino acid sequence set out in SEQ ID NO: 2, which are expected to exhibit anti-bacterial, anti-viral activity or the like especially in human body, and methods in which these molecules are used are provided.

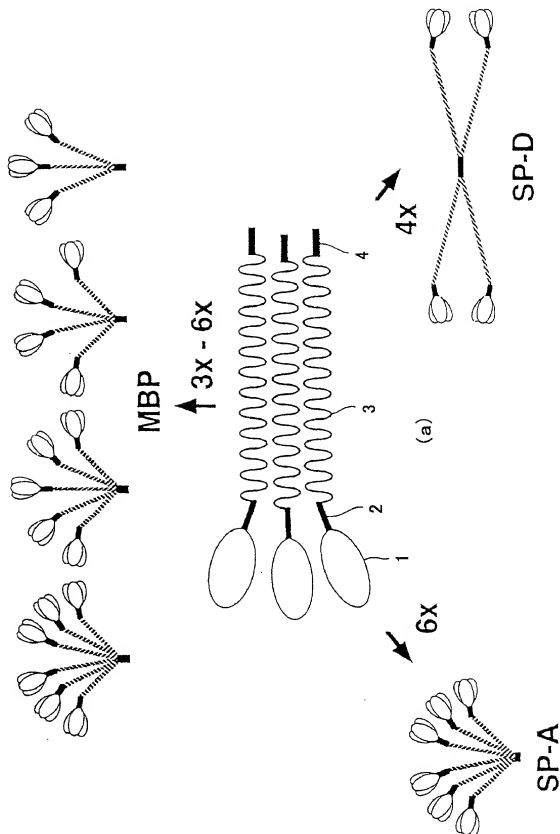
$\frac{1}{11}$ 

Fig. 1

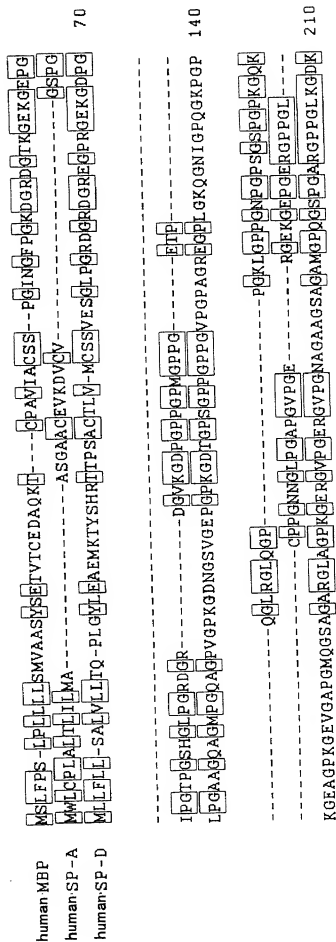


Fig. 2

human MBP  
human SP-A  
human SP-D

GDPG-KSPDQDSSDA-----SERKALQTEMARIKKWLTSLGRQVGNKEFLTNGEIMTFEKV  
--PAHLDEELQATLHD---FRHQILQTRQALS-LQGS-----MTVGERVFSSNGQSIFFDAI  
GIPGKKGAKGFSGLPDVASLRQQQVEALQQQVQHLQAAFSQYKKVELFPNGQSVGKERFKITAGFVKPTEA 260

KALGVKFKQASVATPRNAAENGATQNLH---KEEAFLGITDEKTEGQEVDTIGNRLITYTNWNEGEPNNAGS  
QEACARAGGRINAVPRNPENEALASFAVKKYNITYAYVGLTEGSPGDEPRYSQGITPVNNYTNWYRGEPARG-  
QLCTQAGGQLASPRSA AENALQQLVYAKNEAFLSNITDSKTEGKETYPTEGSLVYSNWA PGEPNDGG 350

DEGCVLLLLNGQWNDVPQSTSRINVCCEPI\*  
KEQCVEMYTPDGQWNDNRNCLYSRLTICEF\*--  
SEDCVEIFINGKWNDRACQCKRLWVCEF\*--

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Fig. 3

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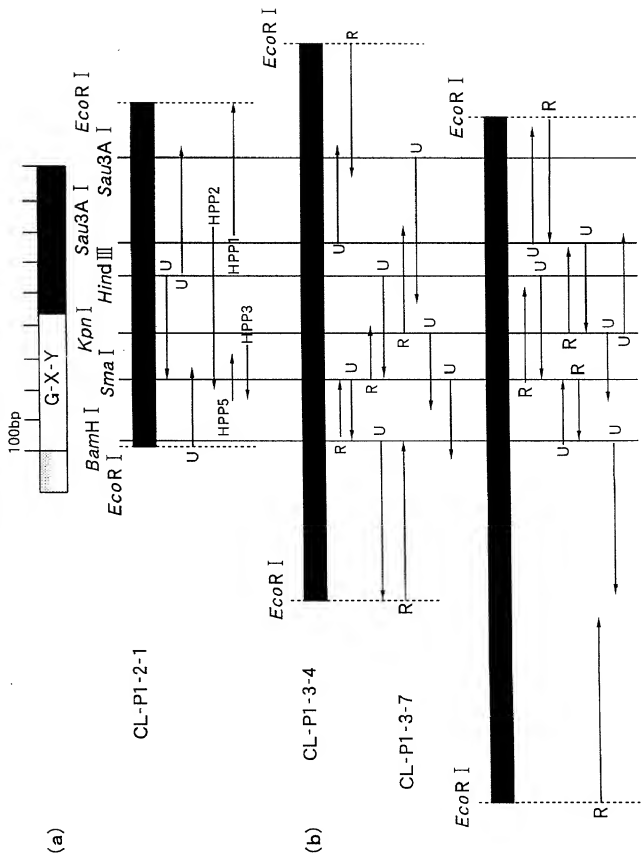


Fig. 4

human MBP  
human SP-A  
human SP-D  
human novel collectin  
70

~~5~~ 1 1

LGLAQLQAAMPGQAGPVCPKQDNCSVTEFGPKKQDTFGSGSPFPSPVPVPAALRECALGKQCQNTIGPQKGP  
 RQGPPTNKQ-----QKGEKPECPGCHGGERSEITGAGPQGERGKSKES---CQPKSGRGSFCK 140

[illegible]

## Final

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P01100.2.F22S200

human MBP  
 human SP-A  
 human SP-D  
 human novel collectin

280  
 350

QSEGHKQKGDPEKSPDG---DSSLASERKALQTEMARIKKWLTFSLG-KQVGNLFFLTNGIMTEK  
 EAEERQH---PQLPA---HLDEELQATLHDFRHQILQTRGALSLOGSIMTVGPEKVFSSNGQSITTEDA  
 HTEGDKAKGESGLPDVASLRQOVEALQGVQHLQAAFSQYKKVLELFENG-QSVGPEKIFKTAGFKVPETTE  
 SPEGHPFH---SCAVVPIALQNEPTPEPDNGC-----HPHWKNTDCCYYFYSVKEIEIFD  
 VVALVKFQASVATPRNAALNGAQNLI---KEEATLQITEKTEGQFVDLITENRLTYTMNNECEENN--  
 IOEADARAGGRIAVPRNPEENEALASFVKKYNTRYAVVLETEGSPGDFRYSQGTIPVNTNMRYRETAG--  
 AQILLTQAGGQIASPRSAAEANAALQOLVNAKMEAAFLSMITDSKTEGKFTYPTIESLVMSMAPEEND--  
 AKLFDKSSHIVINTPEEQWKKQMG-RESHWTGLTDSERINEMKWLDTSPDYKNNKAGQEDNMG

--AGSPEDQVLLKNGOWNIVPCSTSHLAVCEPPI\*-----  
 --RCKEQGVEMYYTGQNNRNGLYSRLTQDF\*-----  
 --DGSSEDQVEIFTNKKNDRAQGEKRLVVCE\*-----  
 HGHCPCEDCAGLIYAGOWNDFQCEDEVNNEICEKDRETIVLSSAL\*

Fig. 6



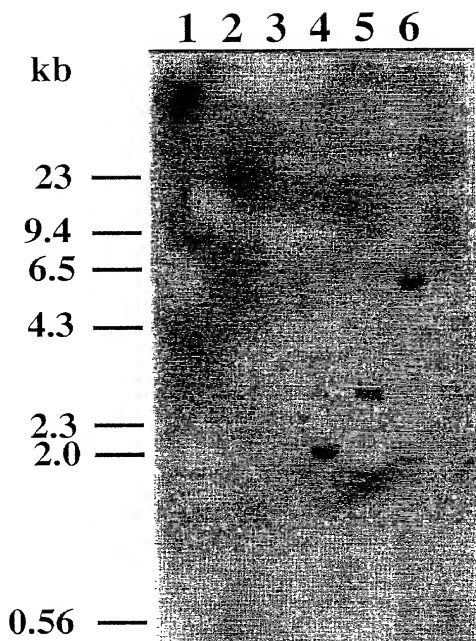
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Fig. 7

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Fig. 8

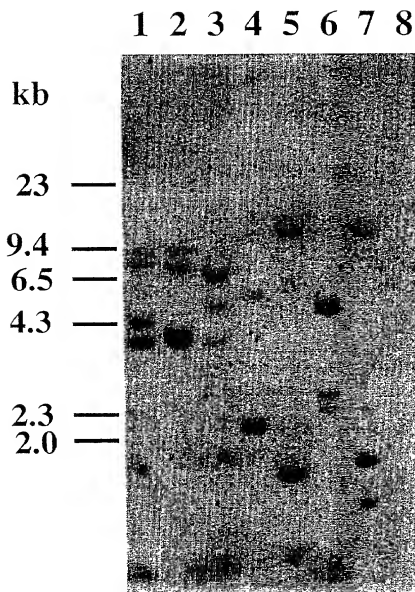
9/  
11

Fig. 9

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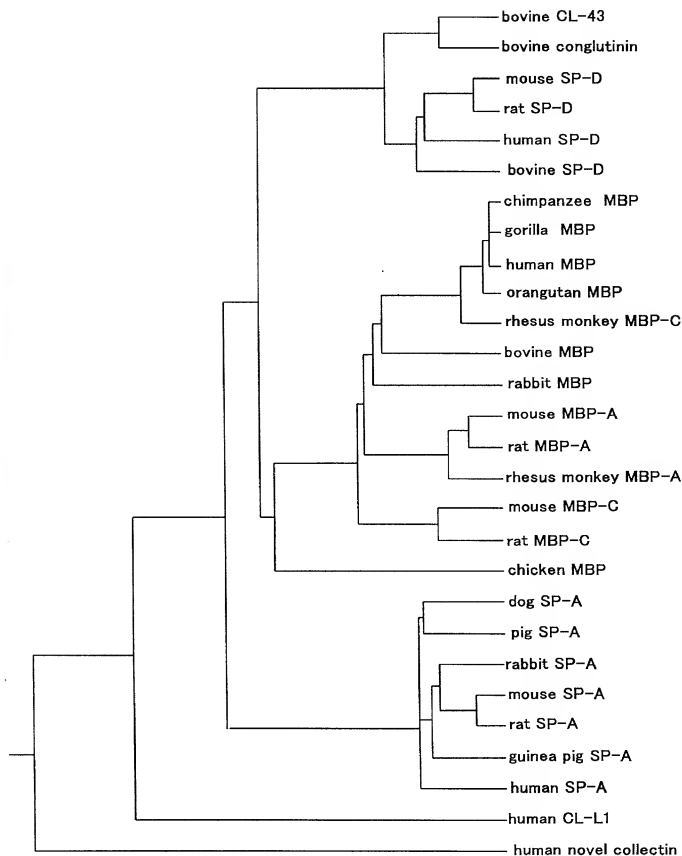


Fig. 10

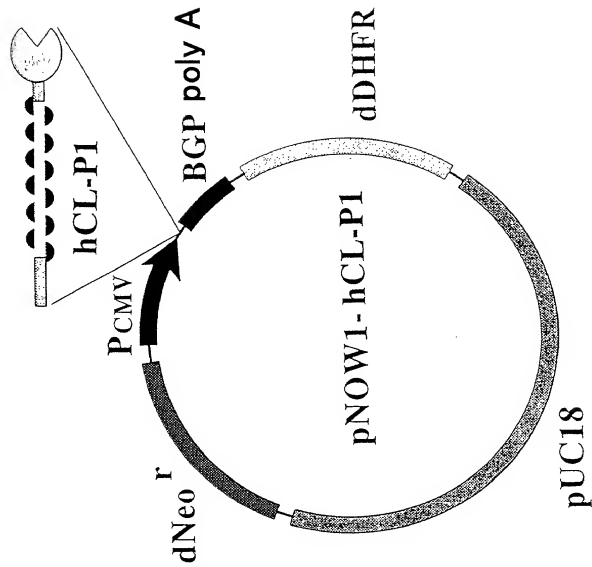


Fig. 11

**DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "NOVEL COLLECTIN," the specification of which was transmitted to the United States Receiving Office on February 26, 2001 as U.S. National Phase of PCT International Application No. PCT/JP99/04452 filed on August 24, 1999 and amended by Preliminary Amendment dated February 26, 2001. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

HEI-10-237611	JAPAN	24 August 1998	Priority Claimed
(Application Serial Number)	(Country)	(Day/Month/Year Filed)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

NONE	
(Application Serial Number)	(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

NONE		
(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith

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Date <b>April 5, 2001</b>	Signature <b>Nobutaka Wakamiya</b>

WO 00/11161

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## SEQUENCE LISTING

&lt;110&gt; FUSO PHARMACEUTICAL INDUSTRIES, LTD.

&lt;120&gt; Novel Collectin

&lt;130&gt; 99P147W0

&lt;150&gt; JP 10-237611

&lt;151&gt; 1998-08-24

&lt;160&gt; 29

&lt;210&gt; 1

&lt;211&gt; 2024

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (670)..(1695)

&lt;400&gt; 1

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aacctcatca cgaatctgca gcggtctgtg gatgacacaa gccaggctat ccagcgaatc	180
aagaacgact ttcaaaatct gcagcaggtt ttcttcaag ccaagaagga cacggattgg	240
ctgaaggaga aatgtcagag ctgtcagacg ctggctgcc acaactctgc gtltggccaaa	300
gccacaacag acaccttgga ggataatgaac agccagctca acatcttacc aggtcagatg	360



gagaacatca ccactatcic tcaagccaac gagcagaacc tgaagacct gcaggactia 420  
 cacaaagaig cagagaatag aacagccaic aagticaacc aactggagga acgcitccag 480  
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 cggacgctga ccagcaatci aaatgaaglc aggaccactt gcacagatac ccttaccaaa 600  
 cacacagaig atcigaccic ctgaaataat accctggcca acatccgttt ggattctgtt 660  
 tccttcagg atg caa caa gat ttg atg agg tcg agg tta gac act gaa gla 711

Met Gln Gln Asp Leu Met Arg Ser Arg Leu Asp Thr Glu Val

1 5 10

gcc aac tta tca glg att atg gaa gaa atg aag cta gla gac tcc aag 759

Ala Asn Leu Ser Val Ile Met Glu Glu Met Lys Leu Val Asp Ser Lys

15 20 25 30

cat ggt cag ctc atc aag aat ttt aca ata cta caa ggt cca ccg ggc 807

His Gly Gln Leu Ile Lys Asn Phe Thr Ile Leu Gln Gly Pro Pro Gly

35 40 45

ccc agg ggt cca aga ggt gac aga gga tcc cag gga ccc cct ggc cca 855

Pro Arg Gly Pro Arg Gly Asp Arg Gly Ser Gln Gly Pro Pro Gly Pro

50 55 60

act ggc aac aag gga cag aaa gga gag aag ggg gag cct gga cca cct 903

Thr Gly Asn Lys Gly Gln Lys Gly Glu Lys Gly Glu Pro Gly Pro Pro

65 70 75

ggc cct gcg ggt gag aga ggc cca att gga cca gct ggt ccc ccc gga 951

Gly Pro Ala Gly Glu Arg Gly Pro Ile Gly Pro Ala Gly Pro Pro Gly

80 85 90

gag cgt ggc ggc aaa gga tct aaa ggc tcc cag ggc ccc aaa ggc tcc 999

Glu Arg Gly Gly Lys Gly Ser Lys Gly Ser Gln Gly Pro Lys Gly Ser

95 100 105 110

cgt ggt tcc cct ggg aag ccc ggc cct cag ggc ccc agt ggg gac cca 1047

Arg Gly Ser Pro Gly Lys Pro Gly Pro Gln Gly Pro Ser Gly Asp Pro

115 120 125

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 130 135 140  
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 Pro Pro Gly Phe Gln Gly Leu Gln Gly Thr Val Gly Glu Pro Gly Val  
 145 150 155  
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 Pro Gly Pro Arg Gly Leu Pro Gly Leu Pro Gly Val Pro Gly Met Pro  
 160 165 170  
 ggc ccc aag ggc ccc ccc ggc cct cct ggc cca tca gga gcg glg gtg 1239  
 Gly Pro Lys Gly Pro Pro Gly Pro Pro Gly Pro Ser Gly Ala Val Val  
 175 180 185 190  
 ccc ctg gcc ctg cag aat gag cca acc ccg gca ccg gag gac aat ggc 1287  
 Pro Leu Ala Leu Gln Asn Glu Pro Thr Pro Ala Pro Glu Asp Asn Gly  
 195 200 205  
 tgc ccg cct cac tgg aag aac ttc aca gac aaa tgc tac tat ttt tca 1335  
 Cys Pro Pro His Trp Lys Asn Phe Thr Asp Lys Cys Tyr Tyr Phe Ser  
 210 215 220  
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 Val Glu Lys Glu Ile Phe Glu Asp Ala Lys Leu Phe Cys Glu Asp Lys  
 225 230 235  
 tct tca cat ctt gtt ttc ata aac act aga gag gaa cag caa tgg ata 1431  
 Ser Ser His Leu Val Phe Ile Asn Thr Arg Glu Glu Gln Gln Trp Ile  
 240 245 250  
 aaa aaa cag atg gla ggg aga gag agc cac tgg atc ggc ctc aca gac 1479  
 Lys Lys Gln Met Val Gly Arg Glu Ser His Trp Ile Gly Leu Thr Asp  
 255 260 265 270  
 tca gag cgt gaa aat gaa tgg aag tgg ctg gat ggg aca tct cca gac 1527  
 Ser Glu Arg Glu Asn Glu Trp Lys Trp Leu Asp Gly Thr Ser Pro Asp

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tac aaa aat tgg aaa gct gga cag ccg gat aac tgg ggt cat ggc cat			1575
Tyr Lys Asn Trp Lys Ala Gly Gln Pro Asp Asn Trp Gly His Gly His			
290	295	300	
ggg cca gga gaa gac tgl gct ggg tlg att tat gct ggg cag tgg aac			1623
Gly Pro Gly Glu Asp Cys Ala Gly Leu Ile Tyr Ala Gly Gln Trp Asn			
305	310	315	
gat ttc caa tgt gaa gac gtc aat aac ttc att tgc gaa aaa gac agg			1671
Asp Phe Gln Cys Glu Asp Val Asn Asn Phe Ile Cys Glu Lys Asp Arg			
320	325	330	
gag aca gta ctg tca tct gca tta taacggactg tgaigggaac acatgagcaa			1725
Glu Thr Val Leu Ser Ser Ala Leu			
335	340		
atttcagct ctcaaaggca aaggacacac ctctctaatt gcatcacctt ctcatcagat			1785
tgaaaaaaaa aaaagcacig aaaaccaait actgaaaaaa aatigacagc tagtgttttt			1845
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&lt;210&gt; 2

&lt;211&gt; 547

&lt;212&gt; PRT

&lt;213&gt; Homo Sapiens

&lt;220&gt;

<223> Deduced Amino Acid Sequence of Novel Collectin from Nucleotide Sequence.

&lt;400&gt; 2

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Met Tyr Ser His Asn Val Val Ile Met Asn Leu Asn Asn Leu Asn Leu  
 1 5 10 15  
 Thr Gln Val Gln Gln Arg Asn Leu Ile Thr Asn Leu Gln Arg Ser Val  
 20 25 30  
 Asp Asp Thr Ser Gln Ala Ile Gln Arg Ile Lys Asn Asp Phe Gln Asn  
 35 40 45  
 Leu Gln Gln Val Phe Leu Gln Ala Lys Lys Asp Thr Asp Trp Leu Lys  
 50 55 60  
 Glu Lys Val Gln Ser Leu Gln Thr Leu Ala Ala Asn Asn Ser Ala Leu  
 65 70 75 80  
 Ala Lys Ala Asn Asn Asp Thr Leu Glu Asp Met Asn Ser Gln Leu Asn  
 85 90 95  
 Ser Phe Thr Gly Gln Met Glu Asn Ile Thr Thr Ile Ser Gln Ala Asn  
 100 105 110  
 Glu Gln Asn Leu Lys Asp Leu Gln Asp Leu His Lys Asp Ala Glu Asn  
 115 120 125  
 Arg Thr Ala Ile Lys Phe Asn Gln Leu Glu Glu Arg Phe Gln Leu Phe  
 130 135 140  
 Glu Thr Asp Ile Val Asn Ile Ile Ser Asn Ile Ser Tyr Thr Ala His  
 145 150 155 160  
 His Leu Arg Thr Leu Thr Ser Asn Leu Asn Glu Val Arg Thr Thr Cys  
 165 170 175  
 Thr Asp Thr Leu Thr Lys His Thr Asp Asp Leu Thr Ser Leu Asn Asn  
 180 185 190  
 Thr Leu Ala Asn Ile Arg Leu Asp Ser Val Ser Leu Arg Met Gln Gln  
 195 200 205  
 Asp Leu Met Arg Ser Arg Leu Asp Thr Glu Val Ala Asn Leu Ser Val  
 210 215 220  
 Ile Met Glu Glu Met Lys Leu Val Asp Ser Lys His Gly Gln Leu Ile

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225	230								235								240			
Lys	Asn	Phe	Thr	Ile	Leu	Gln	Gly	Pro	Pro	Gly	Pro	Arg	Gly	Pro	Arg					
245								250				255								
Gly	Asp	Arg	Gly	Ser	Gln	Gly	Pro	Pro	Gly	Pro	Thr	Gly	Asn	Lys	Gly					
260								265				270								
Gln	Lys	Gly	Glu	Lys	Gly	Glu	Pro	Gly	Pro	Pro	Gly	Pro	Ala	Gly	Glu					
275								280				285								
Arg	Gly	Pro	Ile	Gly	Pro	Ala	Gly	Pro	Pro	Gly	Glu	Arg	Gly	Gly	Lys					
290								295				300								
Gly	Ser	Lys	Gly	Ser	Gln	Gly	Pro	Lys	Gly	Ser	Arg	Gly	Ser	Pro	Gly					
305				310				315				320								
Lys	Pro	Gly	Pro	Gln	Gly	Pro	Ser	Gly	Asp	Pro	Gly	Pro	Pro	Gly	Pro					
325								330				335								
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340								345				350								
Gly	Leu	Gln	Gly	Thr	Val	Gly	Glu	Pro	Gly	Val	Pro	Gly	Pro	Arg	Gly					
355								360				365								
Leu	Pro	Gly	Leu	Pro	Gly	Val	Pro	Gly	Met	Pro	Gly	Pro	Lys	Gly	Pro					
370				375				380												
Pro	Gly	Pro	Pro	Gly	Pro	Ser	Gly	Ala	Val	Val	Pro	Leu	Ala	Leu	Gln					
385				390				395				400								
Asn	Glu	Pro	Thr	Pro	Ala	Pro	Glu	Asp	Asn	Gly	Cys	Pro	Pro	His	Trp					
405								410				415								
Lys	Asn	Phe	Thr	Asp	Lys	Cys	Tyr	Tyr	Phe	Ser	Val	Glu	Lys	Glu	Ile					
420								425				430								
Phe	Glu	Asp	Ala	Lys	Leu	Phe	Cys	Glu	Asp	Lys	Ser	Ser	His	Leu	Val					
435								440				445								
Phe	Ile	Asn	Thr	Arg	Glu	Glu	Gln	Gln	Trp	Ile	Lys	Lys	Gln	Met	Val					
450				455				460												

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Gly Arg Glu Ser His Trp Ile Gly Leu Thr Asp Ser Glu Arg Glu Asn  
 465                                      470                                      475                                      480  
 Glu Trp Lys Trp Leu Asp Gly Thr Ser Pro Asp Tyr Lys Asn Trp Lys  
    485                                      490                                      495  
 Ala Gly Gln Pro Asp Asn Trp Gly His Gly His Gly Pro Gly Glu Asp  
    500                                      505                                      510  
 Cys Ala Gly Leu Ile Tyr Ala Gly Gln Trp Asn Asp Phe Gln Cys Glu  
    515                                      520                                      525  
 Asp Val Asn Asn Phe Ile Cys Glu Lys Asp Arg Glu Thr Val Leu Ser  
    530                                      535                                      540  
 Ser Ala Leu  
 545

&lt;210&gt; 3

&lt;211&gt; 27

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Modified Consensus Sequence of collectins Hybridizable with Novel  
 Collectin.

&lt;400&gt; 3

Glu Lys Cys Val Glu Met Tyr Thr Asp Gly Lys Trp Asn Asp Arg Asn  
    1                                      5                                      10                                      15  
 Cys Leu Gln Ser Arg Leu Ala Ile Cys Glu Phe  
    20                                      25

&lt;210&gt; 4

8/17

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a Reverse Primer for Screening a Novel Collectin.

&lt;400&gt; 4

caatctgatg agaaggtgat g

21

&lt;210&gt; 5

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a Forward Primer for Screening a Novel Collectin.

&lt;400&gt; 5

acgaggggct ggatgggaca t

21

&lt;210&gt; 6

&lt;211&gt; 27

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Consensus sequence of three collectins which were reported heretofore.

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<400> 6

Glu Asp Cys Val Leu Leu Leu Lys Asn Gly Gln Trp Asn Asp Val Pro

1

5

10

15

Cys Ser Thr Ser His Leu Ala Val Cys Glu Phe

20

25

&lt;210&gt; 7

&lt;211&gt; 24

&lt;212&gt; DNA

### ⟨213⟩ Artificial Sequence

 $\langle 220 \rangle$ 

<223> M13 Universal Primer Sequence for Sequencing.

&lt;400&gt; 7

cgacgttgta aaacgacggc cagt

24

&lt;210&gt; 8

&lt;211&gt; 17

&lt;212&gt; DNA

### ⟨213⟩ Artificial Sequence

 $\langle 220 \rangle$ 

<223> M13 Reverse Primer Sequence for Sequencing.

<400> 8

caggaaaca gctatgac

17



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&lt;210&gt; 9

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Sequence of a  $\lambda$ gt11 Reverse Primer for Sequencing.

&lt;400&gt; 9

ttgacaccag accaactggt aatg

24

&lt;210&gt; 10

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Sequence of a  $\lambda$ gt11 Forward Primer for Sequencing.

&lt;400&gt; 10

ggtagcgacg actccaggag cccg

24

&lt;210&gt; 11

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a Primer for Screening a Novel Collectin.

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&lt;400&gt; 11

cgtgaaaaatg aatggaagtg g

21

&lt;210&gt; 12

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a Primer for Screening a Novel Collectin.

&lt;400&gt; 12

ttttatccat tgcgttccct c

21

&lt;210&gt; 13

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a Primer for Sequencing a Novel Collectin.

&lt;400&gt; 13

ctggcagtc ccgaggtcca g

21

&lt;210&gt; 14

&lt;211&gt; 21

&lt;212&gt; DNA

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a Primer for Sequencing a Novel Collectin.

&lt;400&gt; 14

gctgggtcccc ccggagagcg t

21

&lt;210&gt; 15

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a 1RC2 Primer for Cap Site Sequencing.

&lt;400&gt; 15

caaggtacgc cacagcgtat g

21

&lt;210&gt; 16

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a Synthetic TGP1 Primer for Cap Site Sequencing.

&lt;400&gt; 16

tccttcagttt ccctaattccc

20

<210> 17

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Sequence of a 2RC2 Primer for Cap Site Sequencing.

<400> 17

gtacgccaca gcgtatgatg c

21

<210> 18

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Sequence of a Synthetic TGP2 Primer for Cap Site Sequencing.

<400> 18

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21

<210> 19

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Sequence of a Primer for Screening a Novel Collectin.

<400> 19

gaagacaagt ctccaactct tg

22

<210> 20

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Sequence of a Primer for Screening a Novel Collectin.

<400> 20

ctctgagtct gtgaggccga tc

22

<210> 21

<211> 111

<212> DNA

<213> Artificial Sequence

<220>

<223> Sequence of a Probe for Screening a Novel Collectin.

<400> 21

gaagacaagt ctccacatct tgttttcata aacactagag aggaacagca atggataaaa

60

aaacagatgg tagggagaga gagccactgg atcggccctca cagactcaga g

111

<210> 22

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Sequence of a Forward Primer for Screening a Novel Collectin.

<400> 22

gtgccccctgg ccctgcagaa tg

22

<210> 23

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Sequence of a Reverse Primer for Screening a Novel Collectin.

<400> 23

gcataatcacc ctggggaaca ttittag

26

<210> 24

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Sequence of a Sense Primer for Screening  $\beta$ -Actin.

16/17

&lt;400&gt; 24

caagagatgg ccacggctgc t

21

&lt;210&gt; 25

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Sequence of an Antisense Primer for Screening  $\beta$ -Actin.

&lt;400&gt; 25

tccttctgca tctgtcggc a

21

&lt;210&gt; 26

&lt;211&gt; 39

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Sequence of a Sense Primer for Amplifying the Novel Collectin.

&lt;400&gt; 26

aaggaaaaaa gcggccgcatt gcaacaagat ttgatgagg

39

&lt;210&gt; 27

&lt;211&gt; 29

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

<220>

<223> Sequence of a Reverse Primer for Amplifying the Novel Collectin.

<400> 27

gctctagatt ataatgcaga tgacagtac

29

<210> 28

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Sequence of a Sense Primer for Amplifying the Nockout Gene.

<400> 28

atgcaacaag atttgatgag g

21

<210> 29

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Sequence of a Sense Primer for Amplifying the Nockout Gene.

<400> 29

cctacccggt agaattgacc

20



1

## SEQUENCE LISTING

&lt;110&gt; Wakamiya, N.

&lt;120&gt; Novel Collectin

&lt;130&gt; 19036/37157

&lt;150&gt; JP HEI 10-237611

&lt;151&gt; 1998-08-24

&lt;160&gt; 32

&lt;210&gt; 1

&lt;211&gt; 2024

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (670)..(1695)

&lt;400&gt; 1

gtcacgaatc	tgacgaaga	taccagcgtg	ctccagggca	atctgcagaa	ccaaatgtat	60
tctcataatg	tggtcatcat	gaacctcaac	aacctgaacc	tgaccacagt	gcagcagag	120
aacctcatca	cgaatctgca	gcggtctgtg	gatgacacaa	gccagggtat	ccagcgaatc	180
aagaacgact	ttcaaaatct	gcagcaggtt	ttcttcaag	ccaagaagga	cacggatttg	240
ctgaaggaga	aagtgcagag	cttcgagacg	ctggctgcca	acaactctgc	gttggccaaa	300
gccaacaacg	acacccctgga	ggatatgaac	agccagctca	actcattcac	aggtcagatg	360
gagaacatca	ccactatctc	tcaagccaac	gagcagaacc	tgaagacct	gcaggactta	420
cacaaagatg	cagagaatag	aacagccatc	aagttcaacc	aactggaggga	acgcttccag	480
ctctttgaga	cggatatgtg	gaacatcatt	agcaatatca	gttacacagc	ccaccacctg	540
cggacgtgta	ccagcaatct	aaatgaagtc	aggaccactt	gcacagatac	ccttaccaaa	600
cacacagatg	atctgacctc	cttgaataat	accctggcca	acatccgttt	ggattctggt	660
tctctcagg	atg caa caa	gat ttg atg	agg tcg agg	tta gac act	gaa gta	711
	Met Gln Gln Asp Leu Met Arg Ser Arg Leu Asp Thr Glu Val					
	1	5	10			
gcc aac tta tca gtg att atg gaa gaa atg aag cta gta gac tcc aag						759
Ala Asn Leu Ser Val Ile Met Glu Glu Met Lys Leu Val Asp Ser Lys						
15	20	25	30			
cat ggt cag ctc atc aag aat ttt aca ata cta caa ggt cca ccg gcc						807
His Gly Gln Leu Ile Lys Asn Phe Thr Ile Leu Gln Gly Pro Pro Gly						
35	40	45				
ccc agg ggt cca aga ggt gac aga gga tcc cag gga ccc cct gcc cca						855
Pro Arg Gly Pro Arg Gly Asp Arg Gly Ser Gln Gly Pro Pro Gly Pro						
50	55	60				
act gcc aac aag gga cag aaa gga gga aag ggg gag cct gga cca cct						903
Thr Gly Asn Lys Gly Gln Lys Gly Glu Lys Gly Glu Pro Gly Pro Pro						
65	70	75				
ggc cct cgc ggt gag aga ggc cca att gga cca gct ggt ccc ccc gga						951
Gly Pro Ala Gly Glu Arg Gly Pro Ile Gly Pro Ala Gly Pro Pro Gly						
80	85	90				
gag cgt ggc ggc aaa gga tct tct aaa ggc tcc cag gcc ccc aaa ggc tcc						999
Glu Arg Gly Gly Lys Gly Ser Lys Gly Ser Gln Gly Pro Lys Gly Ser						
95	100	105				
cgt ggt tcc cct ggg aag ccc ggc cct cag ggc ccc agt ggg gac cca						1047
Arg Gly Ser Pro Gly Lys Pro Gly Pro Gln Gly Pro Ser Gly Asp Pro						
115	120	125				
ggc ccc ccg ggc cca cca ggc aaa gag gga ctc ccc ggc cct cag ggc						1095
Gly Pro Pro Gly Pro Pro Gly Lys Glu Gly Leu Pro Gly Pro Gln Gly						
130	135	140				

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cct cct ggc ttc cag gga ctt cag ggc acc gtt ggg gag cct ggg gtg 1143
Pro Pro Gly Phe Gln Gly Leu Gln Gly Thr Val Gly Glu Pro Gly Val
145 150 155
cct gga cct cgg gga ctg cca ggc ttg cct ggg gta cca ggc atg cca 1191
Pro Gly Pro Arg Gly Leu Pro Gly Leu Pro Gly Val Pro Gly Met Pro
160 165 170
ggc ccc aag ggc ccc ccc ggc cct cct ggc cca tca gga gcg gtg gtg 1239
Gly Pro Lys Gly Pro Pro Gly Pro Gly Pro Ser Gly Ala Val Val
175 180 185
ccc ctg gcc ctg cag aat gag cca acc cgg gca cca gag gac aat ggc 1287
Pro Leu Ala Leu Gln Asn Glu Pro Thr Pro Ala Pro Glu Asp Asn Gly
195 200 205
tgc ccg cct cac tgg aag aac ttc aca gac aaa tgc tac tat ttt tca 1335
Cys Pro Pro His Trp Lys Asn Phe Thr Asp Lys Cys Tyr Tyr Phe Ser
210 215 220
gtt gag aaa gaa att ttt gag gat gca aag ctt ttc tgt gaa gac aag 1383
Val Glu Lys Glu Ile Phe Glu Asp Ala Lys Leu Phe Cys Glu Asp Lys
225 230 235
tct tca cat ctt gtt ttc ata aac act aga gag gaa cag caa tgg ata 1431
Ser Ser His Leu Val Phe Ile Asn Thr Arg Glu Glu Gln Gln Trp Ile
240 245 250
aaa aaa cag atg gta ggg aga gag agc cac tgg atc ggc ctc aca gac 1479
Lys Lys Gln Met Val Gly Arg Glu Ser His Trp Ile Gly Leu Thr Asp
255 260 265
tca gag cgt gaa aat gaa tgg aag tgg ctg gat ggg aca tct cca gac 1527
Ser Glu Pro His Leu Asn Glu Trp Lys Ser Leu Asp Gly Thr Ser Pro Asp
275 280 285
tac aaa aat tgg aaa gct gga cag cgg gat aac tgg ggt cat ggc cat 1575
Tyr Lys Asn Trp Lys Ala Gly Gln Pro Asp Asn Trp Gly His Gly His
290 295 300
ggg cca gga gaa gac tgt gct ggg ttg att tat gct ggg cag tgg aac 1623
Gly Pro Gly Glu Asp Cys Ala Gly Leu Ile Tyr Ala Gln Trp Asn
305 310 315
gat ttc caa tgt gaa gac gtc aat aac ttc att tgc gaa aaa gac agg 1671
Asp Phe Gln Cys Glu Asp Val Asn Asn Phe Ile Cys Glu Lys Asp Arg
320 325 330
gag aca gta ctg tca tct gca tta taacggactg tgatgggacg acatgagcaa 1725
Glu Thr Val Leu Ser Ser Ala Leu
335 340
attttcagct ctcaaaaggca aaggacactc ctttctaatt gcatcacctt ctcacagat 1785
tgaaaaaaa aaaagcactg aaaaccaatt actgaaaaaa aattgcacgc tagtggtttt 1845
taccatccgt cattacccaa agactttggga actaaaatgt tccccagggt gatatgctga 1905
ttttcatgt gcacatggac tgaatccat agattctctt ccgtagctaa ccgtagcatt 1965
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&lt;210&gt; 2

&lt;211&gt; 547

&lt;212&gt; PRT

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;223&gt; Deduced Amino Acid Sequence of Novel Collectin from Nucleotide Sequence

&lt;400&gt;2

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Met Tyr Ser His Asn Val Val Ile Met Asn Leu Asn Asn Leu Asn Leu
1 5 10 15
Thr Gln Val Gln Gln Arg Asn Leu Ile Thr Asn Leu Gln Arg Ser Val
20 25 30
Asp Asp Thr Ser Gln Ala Ile Gln Arg Ile Lys Asn Asp Phe Gln Asn
35 40 45
Leu Gln Gln Val Phe Leu Gln Ala Lys Lys Asp Thr Asp Trp Leu Lys
50 55 60

```

Glu Lys Val Gln Ser Leu Gln Thr Leu Ala Ala Asn Asn Ser Ala Leu  
 65 70 75 80  
 Ala Lys Ala Asn Asn Asp Thr Leu Glu Asp Met Asn Ser Gln Leu Asn  
 85 90 95  
 Ser Phe Thr Gly Gln Met Glu Asn Ile Thr Thr Ile Ser Gln Ala Asn  
 100 105 110  
 Glu Gln Asn Leu Lys Asp Leu Gln Asp Leu His Lys Asp Ala Glu Asn  
 115 120 125  
 Arg Thr Ala Ile Lys Phe Asn Gln Leu Glu Glu Arg Phe Gln Leu Phe  
 130 135 140  
 Glu Thr Asp Ile Val Asn Ile Ile Ser Asn Ile Ser Tyr Thr Ala His  
 145 150 155 160  
 His Leu Arg Thr Leu Thr Ser Asn Leu Asn Glu Val Arg Thr Thr Cys  
 165 170 175  
 Thr Asp Thr Leu Thr Lys His Thr Asp Asp Leu Thr Ser Leu Asn Asn  
 180 185 190  
 Thr Leu Ala Asn Ile Arg Leu Asp Ser Val Ser Leu Arg Met Gln Gln  
 195 200 205  
 Asp Leu Met Arg Ser Arg Leu Asp Thr Glu Val Ala Asn Leu Ser Val  
 210 215 220  
 Ile Met Glu Glu Met Lys Leu Val Asp Ser Lys His Gly Gln Leu Ile  
 225 230 235 240  
 Lys Asn Phe Thr Ile Leu Gln Gly Pro Pro Gly Pro Arg Gly Pro Arg  
 245 250 255  
 Gly Asp Arg Gly Ser Gln Gly Pro Pro Gly Pro Thr Gly Asn Lys Gly  
 260 265 270  
 Gln Lys Gly Glu Lys Gly Glu Pro Gly Pro Pro Gly Pro Ala Gly Glu  
 275 280 285  
 Arg Gly Pro Ile Gly Pro Ala Gly Pro Pro Gly Glu Arg Gly Gly Lys  
 290 295 300  
 Gly Ser Lys Gly Ser Gln Gly Pro Lys Gly Ser Arg Gly Ser Pro Gly  
 305 310 315 320  
 Lys Pro Gly Pro Gln Gly Pro Ser Gly Asp Pro Gly Pro Pro Gly Pro  
 325 330 335  
 Pro Gly Lys Glu Gly Leu Pro Gly Pro Gln Gly Pro Pro Gly Phe Gln  
 340 345 350  
 Gly Leu Gln Gly Thr Val Gly Glu Pro Gly Val Pro Gly Pro Arg Gly  
 355 360 365  
 Leu Pro Gly Leu Pro Gly Val Pro Gly Met Pro Gly Pro Lys Gly Pro  
 370 375 380  
 Pro Gly Pro Pro Gly Pro Ser Gly Ala Val Val Pro Leu Ala Leu Gln  
 385 390 395 400  
 Asn Glu Pro Thr Pro Ala Pro Glu Asp Asn Gly Cys Pro Pro His Trp  
 405 410 415  
 Lys Asn Phe Thr Asp Lys Cys Tyr Tyr Phe Ser Val Glu Lys Glu Ile  
 420 425 430  
 Phe Glu Asp Ala Lys Leu Phe Cys Glu Asp Lys Ser Ser His Leu Val  
 435 440 445  
 Phe Ile Asn Thr Arg Glu Glu Gln Trp Ile Lys Lys Gln Met Val  
 450 455 460  
 Gly Arg Glu Ser His Trp Ile Gly Leu Thr Asp Ser Glu Arg Glu Asn  
 465 470 475 480  
 Glu Trp Lys Trp Leu Asp Gly Thr Ser Pro Asp Tyr Lys Asn Trp Lys  
 485 490 495  
 Ala Gly Gln Pro Asp Asn Trp Gly His Gly His Gly Pro Gly Glu Asp  
 500 505 510  
 Cys Ala Gly Leu Ile Tyr Ala Gly Gln Trp Asn Asp Phe Gln Cys Glu  
 515 520 525  
 Asp Val Asn Asn Phe Ile Cys Glu Lys Asp Arg Glu Thr Val Leu Ser  
 530 535 540  
 Ser Ala Leu  
 545

<210> 3  
 <211> 27  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Modified Consensus Sequence of collectins Hybridizable with Novel  
 Collectin

<400>3  
 Glu Lys Cys Val Glu Met Tyr Thr Asp Gly Lys Trp Asn Asp Arg Asn  
 1 5 10 15  
 Cys Leu Gln Ser Arg Leu Ala Ile Cys Glu Phe  
 20 25

<210> 4  
 <211> 21  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Sequence of a Reverse Primer for Screening a Novel Collectin.

<400> 4  
 caatctgatg agaaggtgat g 21

<210> 5  
 <211> 21  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Sequence of a Forward Primer for Screening a Novel Collectin.

<400> 5  
 acgaggggct ggatgggaca t 21

<210> 6  
 <211> 27  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Consensus sequence of three collectins which were reported  
 heretofore

<400>6  
 Glu Asp Cys Val Leu Leu Lys Asn Gly Gln Trp Asn Asp Val Pro  
 1 5 10 15  
 Cys Ser Thr Ser His Leu Ala Val Cys Glu Phe  
 20 25

<210> 7  
 <211> 24  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> M13 Universal Primer Sequence for Sequencing

<400> 7  
 cgacgttgta aaacgacggc cagt 24

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<210> 8
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> M13 Reverse Primer Sequence for Sequencing.

<400> 8
caggaaca gctatgac
17

<210> 9
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a lambda gt11 Reverse Primer for Sequencing.

<400> 9
ttgacaccag accaactggt aatg
24

<210> 10
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a lambda gt11 Forward Primer for Sequencing.

<400> 10
ggtggcgacg actcctggag cccg
24

<210> 11
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Primer for Screening a Novel Collectin

<400>11
cgtgaaaatg aatggaagt g
21

<210> 12
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Primer for Screening a Novel Collectin

<400>12
ttttatccat tgctgttct c
21

<210> 13
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Primer for Sequencing a Novel Collectin

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<400>13
ctggcagtc ccgaggtcca g 21

<210> 14
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Primer for Sequencing a Novel Collectin

<400>14
gctgggtcccc ccgagagcgc t 21

<210> 15
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a 1RC2 Primer for Cap Site Sequencing

<400> 15
caaggtacgc cacagcgtat g 21

<210> 16
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Synthetic TGP1 Primer for Cap Site Sequencing

<400> 16
tcttcagttt cctaattccc 20

<210> 17
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a 2RC2 Primer for Cap Site Sequencing

<400> 17
gtacgccaca gcgtatgatg c 21

<210> 18
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Synthetic TGP2 Primer for Cap Site Sequencing

<400> 18
cattcttgac aaacttcata g 21

<210> 19
<211> 22
<212> DNA
<213> Artificial Sequence

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<220>
<223> Sequence of a Primer for Screening a Novel Collectin

<400> 19
gaagacaagt cttcaactct tg                                22

<210> 20
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Primer for Screening a Novel Collectin

<400> 20
ctctgagtct gtgaggccga tc                                22

<210> 21
<211> 111
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Probe for Screening a Novel Collectin

<400> 21
gaagacaagt cttcacatct tgttttcata aacactagag aggaacagca atggataaaa    60
aaacagatgg tagggagaga gagccactgg atcgccctca cagactcaga g          111

<210> 22
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Forward Primer for Screening a Novel Collectin

<400> 22
gtgcccttgg ccttgcagaa tg                                22

<210> 23
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Reverse Primer for Screening a Novel Collectin

<400> 23
gcatatcacc ctggggaaca ttttag                                26

<210> 24
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Sense Primer for Screening Beta Actin

<400> 24
caagagatgg ccacggctgc t                                21

<210> 25

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<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of an Antisense Primer for Screening Beta Actin

<400> 25
tccttctgca tcctgtcggc a 21

<210> 26
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Sense Primer for Amplifying the Novel Collectin.

<400> 26
aaggaaaaaa gcggccgcat gcaacaagat ttgatgagg 39

<210> 27
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Reverse Primer for Amplifying the Novel Collectin

<400> 27
gctctagatt ataatgcaga tgacagtac 29

<210> 28
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Sense Primer for Amplifying the Knockout Gene

<400> 28
atgcaacaag atttgatgag g 21

<210> 29
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence of a Sense Primer for Amplifying the Knockout Gene

<400> 29
cctaccgggt agaattgacc 20

<210> 30
<211> 248
<212> PRT
<213> Homo sapiens

<220>
<223> mannan-binding protein (MBP)

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&lt;400&gt; 30

Met Ser Leu Phe Pro Ser Leu Pro Leu Leu Leu Ser Met Val Ala  
 1 5 10 15

Ala Ser Tyr Ser Glu Thr Val Thr Cys Glu Asp Ala Gln Lys Thr Cys  
 20 25 30

Pro Ala Val Ile Ala Cys Ser Ser Pro Gly Ile Asn Gly Phe Pro Gly  
 35 40 45

Lys Asp Gly Arg Asp Gly Thr Lys Gly Glu Lys Gly Glu Pro Gly Gln  
 50 55 60

Gly Leu Arg Gly Leu Gln Gly Pro Pro Gly Lys Leu Gly Pro Pro Gly  
 65 70 75 80

Asn Pro Gly Pro Ser Gly Ser Pro Gly Pro Lys Gly Gln Lys Gly Asp  
 85 90 95

Pro Gly Lys Ser Pro Asp Gly Asp Ser Ser Leu Ala Ala Ser Glu Arg  
 100 105 110

Lys Ala Leu Gln Thr Glu Met Ala Arg Ile Lys Lys Trp Leu Thr Phe  
 115 120 125

Ser Leu Gly Lys Gln Val Gly Asn Lys Phe Phe Leu Thr Asn Gly Glu  
 130 135 140

Ile Met Thr Phe Glu Lys Val Lys Ala Leu Cys Val Lys Phe Gln Ala  
 145 150 155 160

Ser Val Ala Thr Pro Arg Asn Ala Ala Glu Asn Gly Ala Ile Gln Asn  
 165 170 175

Leu Ile Lys Glu Glu Ala Phe Leu Gly Ile Thr Asp Glu Lys Thr Glu  
 180 185 190

Gly Gln Phe Val Asp Leu Thr Gly Asn Arg Leu Thr Tyr Thr Asn Trp  
 195 200 205

Asn Glu Gly Glu Pro Asn Asn Ala Gly Ser Asp Glu Asp Cys Val Leu  
 210 215 220

Leu Leu Lys Asn Gly Gln Trp Asn Asp Val Pro Cys Ser Thr Ser His  
 225 230 235 240

Leu Ala Val Cys Glu Phe Pro Ile  
 245

&lt;210&gt; 31

&lt;211&gt; 248

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; surfactant protein A (SP-A)

&lt;400&gt; 31

Met Trp Leu Cys Pro Leu Ala Leu Thr Leu Ile Leu Met Ala Ala Ser  
 1 5 10 15

Gly Ala Ala Cys Glu Val Lys Asp Val Cys Val Gly Ser Pro Gly Ile  
                   20                                  25                                  30  
 Pro Gly Thr Pro Gly Ser His Gly Leu Pro Gly Arg Asp Gly Arg Asp  
                   35                                  40                                  45  
 Gly Val Lys Gly Asp Pro Gly Pro Pro Gly Pro Met Gly Pro Pro Gly  
                   50                                  55                                  60  
 Glu Thr Pro Cys Pro Pro Gly Asn Asn Gly Leu Pro Gly Ala Pro Gly  
                   65                                  70                                  75                                  80  
 Val Pro Gly Glu Arg Gly Glu Lys Gly Glu Pro Gly Glu Arg Gly Pro  
                                   85                                  90                                  95  
 Pro Gly Leu Pro Ala His Leu Asp Glu Glu Leu Gln Ala Thr Leu His  
                   100                                  105                                  110  
 Asp Phe Arg His Gln Ile Leu Gln Thr Arg Gly Ala Leu Ser Leu Gln  
                   115                                  120                                  125  
 Gly Ser Ile Met Thr Val Gly Glu Lys Val Phe Ser Ser Asn Gly Gln  
                   130                                  135                                  140  
 Ser Ile Thr Phe Asp Ala Ile Gln Glu Ala Cys Ala Arg Ala Gly Gly  
                   145                                  150                                  155                                  160  
 Arg Ile Ala Val Pro Arg Asn Pro Glu Glu Asn Glu Ala Ile Ala Ser  
                   165                                  170                                  175  
 Phe Val Lys Lys Tyr Asn Thr Tyr Ala Tyr Val Gly Leu Thr Glu Gly  
                   180                                  185                                  190  
 Pro Ser Pro Gly Asp Phe Arg Tyr Ser Asp Gly Thr Pro Val Asn Tyr  
                   195                                  200                                  205  
 Thr Asn Trp Tyr Arg Gly Glu Pro Ala Gly Arg Gly Lys Glu Gln Cys  
                   210                                  215                                  220  
 Val Glu Met Tyr Thr Asp Gly Gln Trp Asn Asp Arg Asn Cys Leu Tyr  
                   225                                  230                                  235                                  240  
 Ser Arg Leu Thr Ile Cys Glu Phe  
                                   245

&lt;210&gt; 32

&lt;211&gt; 375

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; surfactant protein D (SP-D)

&lt;400&gt; 32

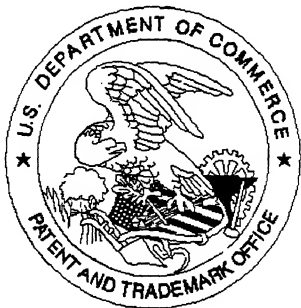
Met Leu Leu Phe Leu Leu Ser Ala Leu Val Leu Leu Thr Gln Pro Leu  
                   1                                  5                                  10                                  15

Gly Tyr Leu Glu Ala Glu Met Lys Thr Tyr Ser His Arg Thr Thr Pro  
                   20                                  25                                  30

Ser Ala Cys Thr Leu Val Met Cys Ser Ser Val Glu Ser Gly Leu Pro  
                   35                                  40                                  45

Gly Arg Asp Gly Arg Asp Gly Arg Glu Gly Pro Arg Gly Glu Lys Gly  
 50 55 60  
 Asp Pro Gly Leu Pro Gly Ala Ala Gly Gln Ala Gly Met Pro Gly Gln  
 65 70 75 80  
 Ala Gly Pro Val Gly Pro Lys Gly Asp Asn Gly Ser Val Gly Glu Pro  
 85 90 95  
 Gly Pro Lys Gly Asp Thr Gly Pro Ser Gly Pro Pro Gly Pro Pro Gly  
 100 105 110  
 Val Pro Gly Pro Ala Gly Arg Glu Gly Pro Leu Gly Lys Gln Gly Asn  
 115 120 125  
 Ile Gly Pro Gln Gly Lys Pro Gly Pro Lys Gly Glu Ala Gly Pro Lys  
 130 135 140  
 Gly Glu Val Gly Ala Pro Gly Met Gln Gly Ser Ala Gly Ala Arg Gly  
 145 150 155 160  
 Leu Ala Gly Pro Lys Gly Glu Arg Gly Val Pro Gly Glu Arg Gly Val  
 165 170 175  
 Pro Gly Asn Ala Gly Ala Ala Gly Ser Ala Gly Ala Met Gly Pro Gln  
 180 185 190  
 Gly Ser Pro Gly Ala Arg Gly Pro Pro Gly Leu Lys Gly Asp Lys Gly  
 195 200 205  
 Ile Pro Gly Asp Lys Gly Ala Lys Gly Glu Ser Gly Leu Pro Asp Val  
 210 215 220  
 Ala Ser Leu Arg Gln Gln Val Glu Ala Leu Gln Gly Gln Val Gln His  
 225 230 235 240  
 Leu Gln Ala Ala Phe Ser Gln Tyr Lys Lys Val Glu Leu Phe Pro Asn  
 245 250 255  
 Gly Gln Ser Val Gly Glu Lys Ile Phe Lys Thr Ala Gly Phe Val Lys  
 260 265 270  
 Pro Phe Thr Glu Ala Gln Leu Leu Cys Thr Gln Ala Gly Gly Gln Leu  
 275 280 285  
 Ala Ser Pro Arg Ser Ala Ala Glu Asn Ala Ala Leu Gln Gln Leu Val  
 290 295 300  
 Val Ala Lys Asn Glu Ala Ala Phe Leu Ser Met Thr Asp Ser Lys Thr  
 305 310 315 320  
 Glu Gly Lys Phe Thr Tyr Pro Thr Gly Glu Ser Leu Val Tyr Ser Asn  
 325 330 335  
 Trp Ala Pro Gly Glu Pro Asn Asp Asp Gly Gly Ser Glu Asp Cys Val  
 340 345 350  
 Glu Ile Phe Thr Asn Gly Lys Trp Asn Asp Arg Ala Cys Gly Glu Lys  
 355 360 365  
 Arg Leu Val Val Cys Glu Phe  
 370 375

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